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Influence of Nutritional Modifications on Sow, Litter, and Nursery Performance

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ABSTRACT

Two separate series of experiments were conducted to determine: 1) the influence of dietary arginine supplementation in late gestation on reproductive performance, and 2) the impact of a whole yeast product in gestation, lactation, and weaned pig diets on reproductive, growth, and immune parameters.

In the first study 99 sows were provided a control diet, or the control diet supplemented with 1% L-arginine from gestation d 93 to 110. Compared to control-fed sows, no differences were observed in reproductive parameters, however a tendency for increased gestational weight gain was observed for sows provided supplemental arginine.

In a second study, 98 sows were provided a control diet, or the control diet supplemented with 0.1 or 0.2% *CitriStim* (whole yeast product, *Pichia guilliermondii* [Pg]) through gestation and lactation. Additionally, two separate nursery studies were conducted in a 3 (sow treatments) × 2 (nursery with or without Pg) factorial arrangement. Pigs were challenged with lipopolysaccharide in a sub-study during the second nursery study. Supplementation with Pg increased number born alive, number weaned, and preweaning mortality, and decreased the number born weighing less than 0.9 kg compared to the control diet. Additionally, total neutrophils and the neutrophil:lymphocyte ratio were increased in sows receiving diets supplemented with Pg. In the first nursery study, pigs from sows fed Pg supplemented diets, but not from control sows, had increased ADG, ADFI, and BW in a linear fashion in phase 1 when Pg was also fed to weaned pigs (interaction; $P < 0.05$). In several instances an additive effect of Pg supplementation to sow diets and nursery diets was observed. Additionally, linear increases in weaned pig growth performance were observed in the second nursery study as the level of Pg inclusion increased in gestation/lactation diets. Supplementation of sow diets with Pg improved

feed intake following LPS challenge in weaned pigs and altered the febrile and immune response compared to pigs from sows fed control diets. In conclusion, Pg in sow and nursery diets improved sow reproductive performance, weaned pig performance, impacted immune parameters and altered immune response to LPS stimulation.

This dissertation is approved for recommendation
to the Graduate Council

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If you are reading this you can safely assume I have passed. It took a little longer than planned, but hopefully you will find the contents of this dissertation as interesting as I. The past few months have been frantic at times, but here we are. Finished!

I want to start off by thanking Dr. Maxwell for broadening my mind in this field far beyond what I ever thought it could be broadened. I know that I have been challenged much more than I had ever anticipated, and have had to drag out knowledge and concepts I never thought I would have to use again. I would like to thank the members of my committee, Drs. Erf, Kegley, and Rosenkrans, for always taking the time to offer help, or confirmation, when needed.

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Thank you to family and friends for your support along the way. Finally, I would like to thank my wife, Anne. I came to Arkansas to get a degree and move on to the next stage of my life. I never thought I would meet you here, and that the next stage of my life would precede the degree, but it could not have worked out better. Thank you for your love and support. I think we should make Isaac call me Dr. Dad.

A four-year-old child could understand this report. Run out and find me a four-year-old child. I can't make head or tail out of it. **~Groucho Marx**

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INTRODUCTION

Increasing input costs will drive pork producers to find new ways to improve efficiency in their production systems and improve profitability. Prolificacy is a fundamental measure of productivity for most livestock operations. In the swine industry, litter size is an economic trait of high importance. An increase of one pig per litter could save pork producers a substantial amount of expense in annual feed costs (Vonnahme et al., 2000). Even a modest 0.2 pig per litter increase could have a large impact on profit, depending on market values (Levis, 2000). While certain parameters are genetically predetermined, many other factors are influenced by nutrition. An understanding of the interactions between factors affecting reproductive performance and nutrition may lead to nutritional programs that would improve fertility and fecundity in livestock species. Additionally, development of maternal feeding strategies that could "program" progeny to enhance performance would be beneficial. The studies presented herein aim to investigate the effect of feeding supplemental L-arginine from gestational day 93 to 110 on sow reproductive, and litter performance. In a separate battery of studies, the effect of supplementation of gestation, lactation, and nursery diets with a whole yeast cell byproduct of citric acid production on performance and immune characteristics was examined.

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Vonnahme, K. A., S. P. Ford, M. E. Wilson, G. R. Foxcroft, G. Gourley, T. Wolf, and M. Quirk-Thomas. 2000. Controls of litter size-Do conclusions drawn from institutional research herds always have relevance to commercial swine production?. Iowa State University Swine Research Report. ASL-R659. Pp. 73-75.

CHAPTER I

REVIEW OF LITERATURE

The research presented in this dissertation deals with the feeding of supplemental L-arginine during gestation on sow reproductive, and litter performance and the effect of supplementing of gestation, lactation, and nursery diets with a whole yeast cell byproduct on performance and immune characteristics. The following review was limited to research conducted in those areas.

L-Arginine

Arginine is considered to be a conditionally essential amino acid in the pig. Under most circumstances, metabolic requirements for arginine are met by endogenous synthesis (Easter et al., 1974). Though arginine synthesis from glutamine has been detected in pig enterocytes prepared within 1 hour of farrowing (Wu and Knabe, 1995), synthesis is not adequate to meet nutrient requirements during the early stages of growth (Southern and Baker, 1983). Synthesis of arginine is potentially insufficient to meet the demands of lactation. Consequently, the diets of rapidly growing swine must contain a source of arginine. However, during postpubertal growth and pregnancy, swine can synthesize arginine at a rate sufficient to meet most or all of their needs (Easter et al., 1974; Easter and Baker, 1976).

Arginine is important in metabolism as a substrate for the synthesis of many proteins. Additionally, arginine is involved in the production of several important metabolites including nitric oxide and polyamines. Nitric oxide is important in the transfer of oxygen and nutrients through the regulation of placental-fetal blood flow (Bird et al., 2003). Additionally, DNA and protein synthesis, and thus cell proliferation and differentiation, are regulated by polyamines

(Flynn et al., 2002). In addition, nitric oxide and polyamines appear to regulate angiogenesis, embryogenesis, and placental and fetal growth (Wu et al., 2004). Thus, arginine is important in establishing and increasing uterine blood flow, as well as the blood supply to the placenta. Increased *Vegfr2* transcriptional activity in fetoplacental tissues was observed in mice during the final third of gestation when provided diets supplemented with L-arginine beginning at breeding (Greene et al., 2012). Arginine can also act as a secretagogue of various hormones, including growth hormone and insulin (Cochard et al., 1998).

The importance of nitric oxide and polyamines on placental and fetal growth has been demonstrated in several studies. Characteristics of intrauterine growth retardation, including reduced weight and size, were observed in endothelial nitric oxide synthase knockout mice due to the absence of nitric oxide (Hefler et al., 2001). When pregnant rats were administered an ornithine decarboxylase inhibitor, which inhibits polyamine production from ornithine, placental weight was reduced and fetal growth was impaired in a manner similar to that observed in intrauterine growth retardation (Ishida et al., 2002). Additionally, supplementation of arginine during gestation prevented fetal growth restriction in hypoxic rats (Vosatka et al., 1998). A decrease in arginine concentration was observed on day 60 of gestation in the fetal plasma, allantoic fluid, and placenta of gilts provided a low protein diet (0.5% CP) compared to control fed (13% CP) gilts (Wu et al., 1998). Rats fed an arginine-free diet, or administered inhibitors of nitric oxide synthesis in their drinking water, had increased resorptions, decreased fetal and placental weight, and fewer live fetus than control rats (Greenberg et al., 1997). Finally, cultured porcine blastocysts significantly depleted arginine from culture medium, likely contributing to nitric oxide and polyamine production (Humpherson et al., 2005). Further, arginine was depleted from the culture of inseminated oocytes (day 0 of culture), two-cell embryos (day 1),

morulae (day 4), and blastocysts (day 6), as well as uninseminated oocytes, indicating the importance of arginine in embryo development (Booth et al., 2005).

Arginase activity is nearly absent in the placenta of the pig, as well as enterocytes of suckling pigs (Wu et al., 1996a; Wu et al. 2005). This helps to maximize arginine supply in the developing pig and neonatal pig from maternal sources. It also causes an increased concentration of arginine in the allantoic fluid of the pig (Wu et al., 1996b). Additionally, concentrations of additional members of the arginine family were increased in porcine allantoic fluid. On gestation day 40, allantoic concentrations of ornithine and glutamine were markedly higher than that of sow serum (Wu et al., 1995; Wu et al., 1996b). Indeed, from gestation day 30 to 40, the concentrations of arginine increase 23-fold, and ornithine and glutamine increase 18- and 4-fold, respectively (Wu et al., 1996b). The increased levels observed are associated with nitric oxide and polyamine synthesis by the placenta during the first half of pregnancy (Wu et al., 2003; Wu et al., 2005).

Analysis of endogenous arginine production of piglets and arginine content of the sow milk they were consuming indicated that the two sources combined provided for daily metabolic need of arginine (Wilkinson et al., 2004). However, in an artificial rearing model supplementation with arginine enhanced piglet performance compared to controls fed to mimic sow milk (Kim et al., 2004). Thus, the combination of endogenous production by the piglet and arginine supplied through the milk diet may not sufficiently supply the metabolic needs of the piglet and may be a limiting factor in maximizing growth.

Arginine Supplementation in Gestation

Genetic selection by the swine industry has improved average litter size, but has not addressed the problem of gestational loss. Additionally, fetal growth rate potential in the modern sows is greater than compared to those of a quarter century ago (McPherson et al., 2004). However, a consequence of this improvement is an apparent negative correlation between litter size and uterine and umbilical blood flow per fetus, which could lead to disproportionate delivery of nutrients to the developing fetuses (Reynolds et al., 1985; Père and Etienne, 2000). As mentioned previously, arginine is involved in angiogenesis in the placenta, as well as growth and development of the fetus. The results of some recent studies have suggested that supplementation with L-arginine may provide benefits to reproductive performance, and performance of the litter during the lactation period.

Early work by Easter and Baker (1976) suggested that gestating gilts did not need supplemental arginine, and even concluded that arginine-free diets could be fed from gestation day 30 through parturition. In their study, no differences were observed in total litter size, number born alive, litter weight of live born pigs or weaning weight (Easter and Baker, 1976), suggesting the gilts in the study were able to provide needed arginine through endogenous synthesis. Additionally, detrimental effects of dietary arginine supplementation have been reported (Li et al., 2010). Li and associates supplemented the gestation diet of gilts with 0.8% L-arginine immediately after breeding through day 25 of gestation. Gilts fed the arginine supplemented diet had reduced uterine weight, total number of corpora lutea, number of total and live fetuses, total fetal weight, and allantoic and amniotic fluid volume when compared to the control, unsupplemented, gilts. This demonstrated that supplementing arginine too early in gestation may impair ovulation rate and embryonic survival in a similar manner as feeding a high

plane of nutrition in early gestation, thus decreasing progesterone concentrations (Li et al., 2010).

In the rat, arginine deficiency during gestation reduced body weight as body reserves may have been mobilized to meet demands for tissue growth (Pau and Milner, 1981). Additionally, placental weight and fetal body weight at term of arginine deficient rats were reduced compared to rats fed the control diet. Pups born to dams fed arginine deficient diets during gestation were lighter than controls, and remained lighter at weaning even when cross fostered on to control fed dams (Pau and Milner, 1981).

However, beneficial effects of arginine supplementation during gestation have also been reported. Supplementation of 1.3% L-arginine to rats throughout gestation did not improve feed intake or maternal body weight gain, but did improve total litter size, number born alive, and litter birth weight when compared to dams fed the control diet (Zeng et al., 2008). Additionally, the same parameters were increased when female rats were provided diets supplemented with arginine from day 1 to day 7 of gestation, and increased inducible nitric oxide and endothelial nitric oxide protein levels at implantation sites. This would indicate a possible improvement in available nitric oxide at implantation sites, and may partially explain the improvement in litter size. It also suggests that different mechanisms are present in early gestation as arginine supplementation from day 0 to day 25 (Li et al., 2010) was detrimental to embryonic survival in gilts.

Not all early supplementation of gestation diets with arginine is detrimental. Gilts provided gestation diets with an arginine supplement (Progenos 28, Trouw Nutrition International, The Netherlands) from gestation day 14 to 28 had more total fetuses and a greater total fetal weight on day 75 of gestation than those provided a control gestation diet (Bérard and

Bee, 2010). Additionally, there were no differences in ovulation rate, average individual fetal body weight, or placenta weight indicating that the improvement in litter size observed on day 75 of gestation was not at the expense of individual pig size. In a similar study with sows, those fed a gestation diet supplemented with Progenos 28 (Trouw Nutrition International) from day 14 to 28 of gestation had 0.8 more pigs per litter in one study, and increased number born alive in gilts (1.08 per litter) and sow (0.93 per litter) in a second study (Ramaekers et al., 2006).

Providing gilts with gestation diets supplemented with 1.0% L-arginine from gestation day 30 through parturition increased number born alive and total litter birth weight, and decreased stillborns when compared to gilts fed an isonitrogenous control gestation diet (Mateo et al., 2007). As in other studies, the improvement in litter size did not come at the expense of average individual piglet body weight. When mixed-parity sows were provided gestation diets supplemented with 1.0% L-arginine from gestation day 22 through parturition, there was an increase in the total number born and born alive, total litter born and born alive birth weight, and total litter born alive placenta weight compared to sows fed the control diet (Gao et al., 2012). In another study, gilts were again provided a control diet, or the control diet supplemented with 1.0% L-arginine from gestation day 30 through parturition. At parturition gilts were provided either the control diet, or arginine supplemented diet through lactation in a 2 x 2 factorial design. Litter size was standardized at farrowing and no effect of gestation diet was observed through the 21 day lactation period (Mateo et al., 2008). However, pigs from sows provided diets supplemented with arginine during lactation had greater body weight at days 7, 14, and 21, and greater average daily gain from day 0 to 7 and day 0 to 21 when compared to those from sows provided the control diet in lactation, but there were no gestation diet × lactation diet interactions (Mateo et al., 2008). Combined, these studies would indicate improvement in reproductive

performance of the sow, and growth performance of the litter, may be realized when both gestation and lactation diets are supplemented with arginine.

Yeast Products

Yeast products have been demonstrated to enhance swine performance as both a probiotic or prebiotic with varied outcomes. Mannan oligosaccharides and β-glucans are components of the yeast cell wall that are believed to confer beneficial effects on immune parameters in vertebrates (Soltanian et al., 2009). These components have been isolated and tested separately on swine. Improvements in growth performance (Davis et al., 2004b) and immune altering effects (White et al., 2002; Davis et al., 2004b) have been demonstrated for mannan oligosaccharide. Immunomodulatory effects of β-glucans have also been demonstrated (Xiao et al., 2004; Soltanian et al., 2009). These benefits may also be conveyed through direct binding of pathogenic bacteria in the intestinal lumen, thereby flushing them out (Spring et al., 2000).

Variance in response to different yeast cell products in weaned pigs may be due to several things including dosage of the product, source of the product, environmental conditions, management techniques, and herd health status. Miguel et al. (2004) suggested that slower growing pigs may benefit most from supplementation of mannan oligosaccharides. Further study is warranted to determine the specific mechanisms through which benefits are conferred by yeast products.

Mannan oligosaccharides influence microbial populations in the intestinal tract by binding to bacteria, preventing them from binding to cells lining the intestine and colonizing (Spring et al., 2000). There is also evidence of an inhibitory effect on lymphocyte function by mannan oligosaccharides (Muchmore et al., 1990; Podzorski et al., 1990), thereby suppressing

immune function and driving nutrients toward gain and away from immune activity (Spurlock, 1997). β-glucan can act as an immunomodulator. In human macrophages β-glucan increased synthesis of IL-1 receptor agonist, however at high levels of β-glucan concentrations of the proinflammatory cytokine IL-1β increased (Poutsiaka et al., 1993). Additionally, the release of TNF-α from mononuclear phagocytes was either enhanced or suppressed dependent on β-glucan concentration (Hoffman et al., 1993).

Yeast Products in Gestation and Lactation

Though not examined in the research presented in this volume, the probiotic form of yeast has been studied as a supplement to gestation and lactation diets. In one such study, active dry yeast was provided as a supplement in the gestation diet of sows beginning at day 93 of gestation and continuing through a 21 day lactation period (Jurgens et al., 1997). Additionally, piglets were provided a starter diet beginning at 12 days of age and continued through weaning. There were no differences in sow body weight, daily feed intake, or reproductive performance in terms of number born alive or litter birth or weaning weights between control fed sows and those provided diets supplemented with active dry yeast (Jurgens et al., 1997). However, milk from sows that were provided the diet supplemented with active dry yeast had an increased concentration of gamma globulin when compared to those receiving the control diet.

Two separate studies were conducted in which mannan oligosaccharide was provided to sows from 4 weeks prior to parturition until 4 weeks following parturition (Czech et al., 2010). The results of the studies were somewhat mixed. In both studies, number of stillborns and preweaning mortality were reduced when sows were provided mannan oligosaccharide in their diet (Czech et al., 2010). In one study only, providing mannan oligosaccharide increased number

born alive and weaned, as well as litter weight at birth and weaning. Concentrations of IgG and IgM in colostrum was greater in sows fed diets containing mannan oligosaccharide compared to those provided the control diet, and IgA tended to be increased (Czech et al., 2010). Additionally, serum concentrations of IgG at birth and 21 days of age, and IgM concentrations at birth were greater in piglets from sows provided mannan oligosaccharide.

Yeast culture is a dried yeast fermentation product derived from *Saccharomyces cerevisiae*, containing the yeast, as well as metabolites from the culture media in which it was grown. Supplementation with yeast culture has been demonstrated to improve milk production and feed intake in ruminants (Robinson and Garrett, 1999; Dann et al., 2000). When fed to sows from day 60 of gestation through day 21 of lactation there was no effect of yeast culture on sow reproductive performance or improvement in nutrient digestibility of the diet (Veum et al., 1995). However, the lysine level fed in the study by Veum contained a low level of total lysine compared to other studies using yeast culture. Additionally, there was a more than 50% reduction, though not statistically significant, in lactation body weight loss in sows provided yeast culture supplemented diets compared to those provided the control diet (5.7 kg and 12.5 kg for yeast culture and control diets, respectively). This may indicate that sows in the trial by Veum and associates (1995), were in a catabolic state due to a nutrient deficiency, and supplementation with yeast culture helped sows to reserve body tissue.

To examine the use of yeast culture in a commercial setting, Kim et al. (2008) top-dressed gestation diets of sows beginning on day 35 of gestation, and continued through lactation. Unlike ruminants, there was not an increase in feed intake by sows provided the diet top-dressed with yeast culture. Additionally, litter size at birth and weaning, and litter birth weight was not different between groups. However, litter weaning weight, daily litter weight

gain, and daily individual piglet gain was increased for sows that were provided the yeast culture top-dressing (Kim et al., 2008). The authors suggested the improvement could be attributed to increased milk production, improved milk quality, mobilization of body reserves by the sow, or improved digestibility of nutrients, however they were not able to measure, or confirm, any of these hypotheses. In an additional study, Kim et al. (2010) investigated whether feeding a constant amount, compared to a constant level of inclusion in the diet, of yeast culture affected the improvement observed in the previously described study (Kim et al., 2008). On day 35 of gestation, sows received either a control, constant percentage of inclusion in the diet of yeast culture, or constant daily amount of yeast culture as a top-dressing, and both yeast culture groups received the same amount of yeast culture as a top-dressing from gestation day 109 through day 21 of lactation. In primiparous sows, providing yeast culture to sows decreased feed intake compared to those fed the control diet, but no differences were observed in reproductive performance (Kim et al., 2010). Additionally, compared to multiparous sows fed the control diet, or diet with constant daily amount of yeast culture, sows fed yeast culture at a constant percentage of inclusion had increased litter weaning weight. Average weight gain of the litter was improved with either yeast culture feeding regimen (Kim et al., 2010).

Finally, the effect of yeast culture supplement fed to sows 5 days prior to breeding, through gestation and lactation, was evaluated by Shen et al. (2011). As in previous studies, body weight was similar between sows fed yeast culture and those fed control diets during gestation and lactation; backfat thickness was also similar during gestation and lactation. Additionally, litter size and average daily feed intake were similar. Similarly to previous studies, there was a tendency for increased litter weaning weight and litter body weight gain from sows that were provided diets supplemented with yeast culture (Shen et al., 2011). In addition to the

improvements in litter performance, a decrease in plasma urea nitrogen was observed on day 110 of gestation in sows provided yeast culture supplemented diets compared to those fed the control diet. No differences in concentration and proportions among peripheral blood leukocytes was observed on day 30 of gestation, but sows fed diets supplemented with yeast culture had lower total leukocyte and neutrophil counts on day 110 of gestation, and lower neutrophil counts again on day 21 of lactation compared to sows fed control diets. The authors speculated that a conditioning period may be required before health and production benefits of yeast culture become apparent (Shen et al., 2011). Additionally, the reduction in plasma urea nitrogen is an indication that sows provided the diet supplemented with yeast culture may be using protein more efficiently in late gestation compared to sows fed the control diet. Improved efficiency, and health status as evidenced by the reduction in circulating neutrophils, may explain some of the improvement in litter weight gain observed for sows provided diets supplemented with yeast culture.

Yeast Products in the Weaned Pig

The abrupt change from milk to solid feed, coupled with a change in environment, place a large amount of stress on young pigs at weaning. The removal of milk at weaning creates an absence of compounds needed by the intestinal epithelium, which may affect intestinal cell growth processes, cell differentiation, cell function, and immune function (Pluske, 2001). Additionally, protections that were provided via immunoglobulins from sow milk are removed, creating susceptibility to pathogens for the weaned pig. During the first week following weaning there can be reduced feed intake, which is detrimental to the intestinal tract of the pig (Dong and Pluske, 2007). The typical growth check observed following weaning may result in mild

malabsorption and increase susceptibility to enteric infection, leading to delayed maturation of intestinal cells. In order to attain optimal digestive and absorptive capacity, the young pig requires a large luminal surface area lined with mature, functional enterocytes. A healthy digestive tract, coupled with a robust immune system should benefit performance of weaned pigs. Yeast cell products, such as mannan oligosaccharides, β-glucans, and yeast culture have been examined, with variable results, to aid in the transition the weaned pig faces.

Mannan oligosaccharides. Numerous studies have examined the effects of mannan oligosaccharides derived from *Saccharomyces cerevisiae* on weaned pig performance when provided during the nursery period. When mannan oligosaccharides were supplemented to nursery diets an increase in average daily gain and gain:feed ratio was observed in the initial (0 to 14 day) and overall (0 to 21 day) nursery periods, and body weight was greater on days 14 and 21 when compared to control fed piglets (Davis et al., 2004b). Additionally, weaned pigs that were provided mannan oligosaccharides in nursery diets had a greater percentage of lymphocytes, and lower percentage of neutrophils in the leukocyte population than pigs provided the control diet, leading the authors to conclude that providing mannans to weaned pigs reduced the inflammatory response often observed at weaning. The response to mannan oligosaccharides was varied among farms when a study was carried out on three separate farms to determine the effect of mannans on weaned pig growth performance (Rozeboom et al., 2005). Average daily gain was consistently greater when weaned pigs were provided nursery diets supplemented with mannan oligosaccharides during day 11 to 42 and the overall 42 day study when compared to those fed the control diet, though the magnitude of the response varied among the 3 farms. Additionally, mannan oligosaccharides increased growth rate more from day 11 to 42 when an

antimicrobial was not included in the diet. An improvement in gain:feed ratio from day 11 to 42 and the overall 42 day study was observed when mannans were supplemented in the nursery diets, but only on 1 of the 3 farms (Rozeboom et al., 2005). In yet another study, weaned pigs provided mannans had a greater average daily gain and average daily feed intake from day 0 to 14, but similar gain:feed ratio, when compared to weaned pigs fed a control diet (Zhao et al., 2012).

No improvements were observed in average daily gain, average daily feed intake, or the gain:feed ratio when mannan oligosaccharides were supplemented in the diets of weaned pigs when compared to those that received a control diet (Nochta et al., 2009; Nochta et al., 2010; Che et al., 2012). However, Nochta and associates (2010) reported that supplementation with mannans increased the ileal digestibility of lysine, methionine, cysteine, and threonine, as well as calcium and phosphorus. Tumor necrosis factor alpha production was lower, and interleukin-10 production was increased in alveolar macrophages from weaned pigs that were fed mannan oligosaccharides compared to control fed weaned pigs when stimulated with lipopolysaccharide (Che et al., 2012).

Nursery diets are often supplemented with pharmacological addition of copper and zinc due to the improvement in health and growth performance they provide (Stahly et al., 1980; Hill and Spears, 2001; Maxwell and Carter, 2001). An increase in average daily gain and gain:feed ratio from day 0 to 10 in the absence of additional supplemental copper was observed when pigs were weaned at 18 days of age and provided diets supplemented with mannan oligosaccharide (Davis et al., 2002). Additionally, an increase was observed in the same traits from day 24 to 38, and day 0 to 38 when mannan oligosaccharide was provided in the nursery diet, regardless of copper supplementation. Immune response as measured by *in vitro* lymphocyte proliferation

was not affected by the addition of mannan oligosaccharide to nursery diets (Davis et al., 2002). In a series of experiments by LeMieux et al. (2003), inclusion of mannan oligosaccharides in the diet of weaned pigs was examined in the presence or absence of pharmacological levels of zinc. There was no improvement in the performance of weaned pigs when mannan oligosaccharides were provided in the diet in the presence of pharmacological levels of zinc. However, in the absence of zinc, weaned pigs provided diets supplemented with mannan oligosaccharides had increased average daily feed intake from day 7 to 21 and 0 to 28 of the nursery study, average daily gain from day 21 to 28 and 0 to 28, and gain:feed from day 7 to 21 and 21 to 28 (LeMieux et al., 2003). In a separate study reported in the same manuscript, the variability of mannan oligosaccharides on performance was demonstrated as average daily feed intake was reduced by mannan oligosaccharides in the diet from day 0 to 21 in contrast to the previous study, but average daily gain and gain:feed ratio remained greater though the effect was observed earlier, from day 7 to 21 and the overall 21 day nursery study. Combined these studies demonstrate a benefit of mannan oligosaccharides during phase 2 (day 7 to 21) of the nursery period in the absence of pharmacological levels of zinc. Variable responses were again observed by Davis et al. (2004a) when mannan oligosaccharides were provided in nursery diets with, and without, pharmacological levels of zinc oxide. No growth or lymphocyte proliferation responses to mannan oligosaccharides were noted in the first experiment. However, pigs provided diets supplemented with mannan oligosaccharides had improved average daily gain and gain:feed ratio during the first 10 days of a second nursery study compared to those not provided mannan oligosaccharides (Davis et al., 2004a). In an additional study, supplementation with mannan oligosaccharides decreased lymphocyte proliferation in unstimulated and phytohemaglutinin (nonspecific stimulation of T lymphocytes)-stimulated cells, and mannan oligosaccharides

decreased the proliferation of lymphocytes when pigs were provided 200 ppm zinc compared to those fed diets without mannan oligosaccharides, but no differences were observed when zinc was provided at higher levels (Davis et al., 2004a). Mannan oligosaccharides, or a combination of mannan oligosaccharides and organic zinc, increased gain:feed from postweaning weeks 2 and 5 and in the overall 5 week nursery period (Castillo et al., 2008).

β-glucans. Studies in which β-glucans were supplemented in nursery diets have also had varied results with regard to performance and immune response. No differences were reported in average daily gain or average daily feed intake when pigs were provided prestarter diets supplemented with β-glucans at 7 days of age through weaning and then fed a nursery diet supplemented with β-glucans for 4 weeks postweaning compared to those fed a control diet during the same period of time (Sauerwein et al., 2007). Phagocytic activity of neutrophils and serum concentration of haptoglobin were also similar between pigs provided diets supplemented with β-glucans and those fed the control diet (Sauerwein et al. 2007).

A quadratic effect of β-glucan inclusion in the diet of weaned pigs was reported during day 14 to 28 and 0 to 28 for average daily gain with the greatest numerical improvement at 50 ppm (range 0, 25, 50, 100, and 200 ppm) with no difference observed in average daily feed intake or gain:feed (Li et al., 2006). In a follow up study using the highest level of β-glucan inclusion that elicited a positive response in the previous study (50 ppm), average daily gain and average daily feed intake were increased compared to weaned pigs provided a control diet. Lymphocyte proliferation in response to concanavalin A was reduced in cells cultured from pigs that were fed diets supplemented with β-glucan on day 14 of the nursery study, but no difference was reported on day 28 (Li et al., 2006). Additionally, Li and associates (2006) challenged pigs

with lipopolysaccharide on day 31 of the nursery study and pigs that were provided diets supplemented with β-glucan had lower plasma IL-6 at 1.5, 3, and 4.5 hours post-challenge, lower TNF-α concentrations at 3 and 4.5 hours post-challenge, and increased IL-10 concentrations at 3, 4.5, 6, and 7.5 hours post-challenge. This suggests β-glucan inclusion in nursery diets may alter the immune response to lipopolysaccharide, promoting the secretion of anti-inflammatory cytokines and limiting the secretion of proinflammatory cytokines which could improve growth performance, as observed in the previous study. Similarly, a decrease in plasma IL-6 and increase in IL-10 in response to lipopolysaccharide was reported when weaned pigs were provided diets supplemented with β-glucan (Li et al., 2005). The authors also noted a decrease in IL-6 production by lipopolysaccharide stimulated mononuclear cells from pigs that had been provided diets supplemented with β-glucan. Hiss and Sauerwein (2003) found no differences in lymphocyte proliferation or serum haptoglobin concentration between pigs fed a control diet, or the control diet supplemented with β-glucan. However, there was an increase in serum haptoglobin concentration in pigs with slower growth rates (100 to 200 grams/day) compared to those with high growth rates (greater than 301 grams/day), and a generally negative correlation between serum haptoglobin concentration and growth rate (Hiss and Sauerwein, 2003).

Yeast culture. Yeast culture is a fermented yeast product that is dried, and contains the yeast as well as the metabolites produced by the yeast in the fermentation process. The gain:feed ratio was decreased in pigs provided diets containing soy or peanut hulls, however, supplementation with yeast culture returned gain:feed to the level of pigs fed the control diet during a 5 week nursery study (Kornegay et al., 1995). Yeast culture had no effect on any performance parameter when supplemented in nursery diets containing dried whey, indicating it

may improve fiber digestibility in pigs. Improvements in average daily gain during phase 2 and the overall nursery period, and gain:feed in phase 1, phase 2, and the overall nursery period with similar average daily feed intake were reported in weaned pigs provided a diet supplemented with yeast culture compared to those provided a control diet (van der Peet-Schwering et al., 2007). Shen et al. (2009) also reported improvement in average daily gain and average daily feed intake in pigs provided a diet supplemented with yeast culture. Additionally, yeast culture supplementation increased the apparent digestibility of dry matter, gross energy, and crude protein.

In response to a *Salmonella* challenge, pigs provided diets supplemented with yeast culture had increased average daily gain, and decreased rectal temperature, post-*Salmonella* challenge than those fed a control diet devoid of yeast culture (Price et al., 2010). However, no differences were observed prior to challenge in average daily gain, body weight, gain:feed, or average daily feed intake between treatment groups (Price et al., 2010). Prior to an immune challenge with *Escherichia coli* K88, there were no differences in any performance parameter, however, on day 3 and 7 post-challenge, average daily feed intake was increased in pigs receiving diets supplemented with yeast culture when compared to those receiving the control diet (Kiarie et al., 2011).

CitriStim

CitriStim (ADM, Decatur, IL) is a yeast culture (*Pichia guilliermondii*) byproduct of citric acid production produced using a controlled fermentation process. The product is a source of mannan-oligosaccharides and β-glucans, and contains the whole yeast cell and its components. No benefit to body weight, feed intake, or feed efficiency was reported when *CitriStim* was

provided in broiler diets for 5 weeks (Shanmugasundaram and Selvaraj, 2012). However, on day 21 and 35 of age, broilers fed a diet supplemented with *CitriStim* had a greater number of regulatory T cells, increased IL-10 mRNA, and decreased IL-1β mRNA in the cecal tonsil than those provided a control diet. No differences in these parameters were observed in the spleen, a systemic immune organ, suggesting a localized immunomodulatory effect rather than systemic. In all, supplementation with *CitriStim* appeared to elicit a localized antiinflammatory response in the broiler. As *CitriStim* is a fermented yeast product, it is believed to elicit its effects through mannan oligosaccharides and β-glucans found in the cell wall, and potentially intracellular components and metabolites produced during the controlled fermentation process. Several internal studies have demonstrated an improvement in growth performance and feed efficiency in weaned pigs.

In summary, arginine supplementation during gestation elicits varied responses in reproductive performance of the sow dependent on the timing of the supplementation. Providing arginine in gestation diets as early as 14 days improved litter size. Additionally, improvements in sow reproductive performance and growth and immune performance of weaned pigs has been observed in response to supplementation of diets with prebiotic yeast products. Thus, the aim of the research presented in this volume was two-fold. First, to evaluate the effect of late gestation supplementation with dietary arginine on reproductive and lactation performance. And secondly, to evaluate the effect of supplementation of a whole yeast product, *CitriStim*, in gestation, lactation, and nursery diets on reproductive performance, lactation performance, and weaned pig performance.

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CHAPTER II
INFLUENCE OF DIETARY L-ARGININE SUPPLEMENTATION TO SOWS DURING LATE GESTATION ON SOW AND LITTER PERFORMANCE DURING LACTATION

Abstract

Ninety-nine gilts and sows (PIC GPK35) were used in this study to evaluate the effects of feeding 1% added L-arginine during the final 3 wk of gestation on sow and litter performance. Sows were allotted to one of two dietary treatments, a control diet with no additional L-arginine (CON), or a diet providing an additional 25 g/d L-arginine (ARG), based on parity (0, 1, or 2+) and weight at the initiation of the study (d 93 gestation). From d 93 of gestation to farrowing, animals were provided 2.72 kg feed/d containing 0.63% standard ileal digestible lysine and 3.3 Mcal/kg ME (as-fed) in two equal meals. Control sows were consuming 20.4 g/d and ARG sows were consuming 47.2 g/d of arginine. On d 110, animals were weighed and moved into farrowing. A common lactation diet was provided upon farrowing crates. Sows were then weighed 48 h post-farrowing and at weaning. Late gestational BW gain (d 93 to 110), farrowing (d 110 to 48 h post-farrowing) and lactation BW loss (d 110 to weaning) were calculated. Sow and litter performance data, including number born alive, number weaned, individual birth and weaning weight, and placenta weight were recorded. Plasma samples were collected on d 93 and 110 and IGF-1, insulin, and blood urea nitrogen concentrations analyzed. There was a tendency for a greater late gestation BW gain ($P = 0.06$) in ARG compared to CON (14.4 and 12.0 kg, respectively). Additionally, a tendency for a parity by treatment interaction was observed for late gestation BW gain with parity 1 ARG gaining the most weight, parity 0 ARG with intermediate weight gain, and all other treatments having the lowest weight gain ($P = 0.1$). There were no differences between treatment groups observed in farrowing or lactation BW loss, number born alive, number weaned, birth weight, weaning weight, or placenta weight ($P > 0.33$).

Additionally, there were no differences between CON and ARG gilts and sows for plasma IGF-1, insulin, or BUN concentration at d 93 or 110 of gestation ($P > 0.28$). In conclusion, late gestational supplementation with L-arginine may be beneficial to sow BW gain, independent of litter and placental weight, but had no effect on litter size or lactation performance.

Introduction

By means of genetic selection, the swine industry has improved not only average litter size, but also fetal growth rate potential in modern sows compared to those a quarter century ago (McPherson et al., 2004). However, a consequence of this improvement is an apparent negative correlation between litter size and uterine and umbilical blood flow per fetus, which could lead to disproportionate delivery of nutrients to the developing fetuses (Reynolds et al., 1985; Père and Etienne, 2000). One potential solution to these issues is the use of supplemental dietary L-arginine (L-Arg) in gestation diets of gilts and sows.

Arginine is important in metabolism as a substrate for protein synthesis. Additionally, arginine is a precursor of several important metabolites, including nitric oxide and polyamines. Nitric oxide may be important in its capacity to enhance blood flow, while polyamines are involved in embryogenesis and placental development, and both are involved in angiogenesis (Wu et al., 2004). Increased *Vegfr2* transcriptional activity in fetoplacental tissues was observed in mice during the final third of gestation when provided diets supplemented L-Arg beginning at breeding (Greene et al., 2012). Arginine can also act as a secretagogue of various hormones, including growth hormone and insulin (Cochard et al., 1998). Interestingly, arginine is found in high concentrations in allantoic fluid in early gestation (Wu et al., 1996).

Several studies in which gestation diets have been supplemented with L-Arg have had varying outcomes dependent on the time of supplementation during gestation. Li and associates (2010) observed a reduction in uterine weight, total number of fetuses, number of corpora lutea, total fetal weight, and amniotic and amniotic fluid volume at d 25 of gestation when sows were provided an additional 0.8% L-Arg immediately following breeding to d 25 of gestation. In contrast, gilts fed a gestation diet supplemented with 1.0% L-Arg from d 30 of gestation until parturition had more piglets born alive, an increased litter birth weight of piglets born alive, decreased number of piglets born dead, and decreased plasma urea nitrogen concentrations at gestation d 90 and 110 when compared to controls (Mateo et al., 2007). Finally, supplementation of gestation diets from d 14 to 28 of gestation with an additional 25 g/d L-Arg resulted in an increase of approximately one pig per litter (Ramaekers et al., 2006) in one study, and an increase in total number, and weight, of fetuses at d 75 of gestation (Bérard and Bee, 2010) in another. Supplementation of L-Arg in early gestation appears to have a beneficial effect on litter performance, but it is unclear if there is an optimal time, duration, or level of arginine supplementation during gestation. The objective of this study was to determine the effects of late gestation supplementation of 1.0% L-Arg to a corn, soybean meal based gestation diet on sow and litter performance during lactation.

Materials and Methods

***Animals and Experimental Diets*.** Animal management and experimental procedures conducted during this study were approved by the University of Arkansas Institutional Animal Care and Use Committee.

A study was conducted using 3 consecutive groups of gestating gilts and sows (n = 99) to determine the effects of late gestational supplementation of L-Arg on dam and litter performance. At d 93 of gestation, gilts and sows were individually weighed and allotted, based on BW and parity (0, 1, or 2+), to 1 of 2 dietary treatments consisting of corn, soybean meal, and distillers dried grains with solubles-based diets supplemented with either 1.0% L-Arg·HCl (ARG) or 2.05% L-Ala (isonitrogenous control (CON); Ajinomoto Heartland, Chicago, IL; **Table 1**). Gilts and sows were provided 2.72 kg/d during the test period. The CON diet contained 0.75% standard ileal digestible arginine which provided 20.4 g/d arginine and exceeded NRC (1998) suggested requirements for gestating sows. The supplemental level of 1.0% L-Arg was chosen based on beneficial effects on reproductive parameters in other studies of L-Arg supplementation of gestation diets (Ramaekers et al., 2006; Mateo et al., 2007; Bérard and Bee, 2010) and provided an additional 25 g/d of arginine during the test period. The isonitrogenous control was supplemented with L-Ala as it is not involved in arginine synthesis, not toxic, and catabolized by pigs (Kohli et al., 2004). Gilts and sows were individually housed in gestation crates (0.61 m x 2.13 m) with partially slatted floors until d 110 of gestation when they were individually weighed and transferred to individual farrowing crates (1.22 m x 2.13 m). Upon parturition, sows were fed a common lactation diet through the lactation period. Cross-fostering occurred within treatment groups and occurred within 24 h of farrowing.

Gestation diets contained 3.3 Mcal/kg ME and 18.7% CP, while the lactation diet contained 3.4 Mcal/kg ME and 17.6% CP (as-fed). Diets were formulated to meet or exceed NRC requirements, including arginine, for gestating and lactating gilts and sows (NRC, 1998). A common gestation diet was fed once daily (2.26 kg/d) from d 0 to 93 of gestation. During the test period from d 93 of gestation until farrowing, gestation treatment diets were fed twice daily

(2.72 kg/d total). Lactation diets were provided ad libitum. Water was available for ad libitum intake throughout gestation and lactation.

Individual body weight was recorded for gilts and sows at d 93 and 110 of gestation, 48 h post farrowing, and at weaning. At farrowing, and again at weaning, the total number of live piglets was counted and individual body weight was recorded. Additionally, the number and total weight of stillborns and mummies and total placenta weight were recorded at farrowing. Individual feed intake of each sow was recorded during lactation.

Blood samples were obtained via jugular venipuncture on d 93 and 110 of gestation using K_2EDTA-coated tubes (BD Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ). Blood samples were centrifuged at 1300 x *g* for 20 min. Plasma was transferred into 5.0 mL polypropylene sample tubes and stored at -20˚C until analyzed for IGF-1, insulin, and urea nitrogen concentrations.

Chemical Analysis. Plasma samples were analyzed for IGF-1 using a commercial radioimmunoassay kit according to the manufacturer's instructions (IGF-R21, ALPCO Immunoassays, Salem, NH) with a detection limit of 0.156 ng/mL. Briefly, non-specific binding (NSB), reference (Bo), and total count tubes were prepared. Additionally, 100 µl of prepared standard (range 0.156 to 10 ng/mL) or diluted plasma samples were added to appropriate tubes in duplicate. Next, 100 µl of primary antibody was added to all tubes except total count and NSB. Then, 100 µl of ^{125}I-IGF-1 was added to all tubes and tubes were incubated at 4˚C for 48 h. Following incubation, 500 µl of cold precipitating reagent was added to all tubes, except total count, and allowed to incubate at 4˚C for 1 h. Next, 1.0 mL ice-cold distilled water was added followed by centrifugation at 3000 x *g* for 30 min. Supernatant was removed and activity of

each tube was counted for 1 min using a Wallac 1470 Wizard automatic gamma counter (Wallac Oy, Turku, Finland).

Plasma insulin was determined using a commercial radioimmunoassay kit according to the manufacturer's instructions (PI-12K; Millipore Corp., Billerica, MA) with a detection limit of 2 µU/mL. Briefly, 300, 200, or 100 µl of assay buffer was added to NSB, Bo, and sample tubes, respectively, in duplicate. In duplicate, 100 µl of standards (range 2 to 200 µU/mL) or plasma were added to tubes. Next, 100 µl of ^{125}I-insulin was added to all tubes followed by 100 µl of porcine insulin antibody to all but total count and NSB tubes. Following an overnight incubation at 4˚C, 1.0 mL of cold precipitating reagent was added to all but total count tubes and tubes were incubated for 20 min at 4˚C. All tubes, except total count, were then centrifuged at 3000 x g for 20 min. Supernatant was removed and activity of each tube was counted for 1 min each using a Wallac 1470 Wizard automatic gamma counter.

Plasma urea nitrogen was determined using a colorimetric kit (Urea Nitrogen (BUN) Reagent; Teco Diagnostics, Anaheim, CA). Briefly, samples and standards were diluted using PBS and 5.0 µl of each standard or sample was loaded into duplicate wells of a 96-well microplate. Next, 150 µl of reconstituted BUN enzyme reagent was added to each well and gently mixed. Wells were allowed to incubate for 10 min at room temperature. Following incubation, 150 µl of BUN color developer was added to each well and the plate was gently mixed. Following an additional incubation at room temperature for 10 min, microplates were read at an absorbance of 630 nm on a SpectraMAX 250 microplate reader (Molecular Devices, Sunnyvale, CA). A standard curve was constructed and the concentration of each sample was determined using SOFTmax PRO microplate data acquisition and analysis software (Molecular Devices).

Statistical Analysis. Data were analyzed using the PROC MIXED procedures of SAS with treatment as the fixed effect and parity as a covariate.

Results

Sow performance. Data from 1 animal per treatment group was excluded from the analysis due to illness. Body weight was not recorded for 2 animals per treatment on d 93 of gestation, 1 animal in the CON group on d 110 of gestation, and 3 animals from the ARG group at weaning.

There was a tendency for increased late gestation body weight gain in gilts and sows fed the diet supplemented with arginine compared to those fed the control diet between d 93 and 110 with arginine supplemented gilts and sow gaining approximately 2 kg more than controls (P = 0.06; Figure 1). There was also a tendency (P = 0.1) for a gestation treatment by parity interaction. A greater increase was observed in late gestation body weight gain in parity 0 and parity 1 gilts and sows fed the diet supplemented with 1% arginine from d 93 to d 110 of gestation than those of the control group (P = 0.1; Figure 2). However, no differences were observed in body weight loss following farrowing, or from farrowing to weaning between gilts and sows fed a control diet or a diet supplemented with 1% L-Arginine. Additionally, there were no differences in ADFI, number born alive, number weaned, individual birth weight and weaning weight, number of stillborns and mummies, or total stillborn, mummy, or placenta weight (Table 2).

IGF-1, insulin, and urea nitrogen. Plasma concentrations of IGF-1, insulin, and urea nitrogen on d 93 and 110 of gestation were similar between gilts and sows fed either a control or arginine supplemented late gestation diet (Table 3).

Discussion

Arginine is considered to be a conditionally essential amino acid in the pig. Endogenous synthesis is sufficient to meet arginine requirements in adult animals, but there may be a dietary requirement in younger animals for maximal growth (Southern and Baker, 1983). Previous research has demonstrated that dietary supplementation with L-arginine, as early as d 14 of gestation, may have beneficial effects on sow reproduction and performance of the litter during lactation (Ramaekers et al., 2006; Mateo et al., 2007; Bérard and Bee, 2010).

In the current study, there was a tendency for L-Arg supplemented gilts and sows to gain more weight between d 93 and 110 of gestation than control fed animals. Other studies have reported no differences in late gestation or parturition BW between control and L-Arg supplemented gilts (Mateo et al., 2007, Mateo et al., 2008) or rats (Zeng et al., 2008). There was also a tendency in the current study for first and second litter dams fed the gestation diet supplemented with L-Arg to gain more weight than control fed dams of the same parity. Interestingly, there was no difference in late gestation BW gain between control and arginine supplemented parity 2+ sows. After d 69 of gestation the protein demands for maternal maintenance, fetal tissue, and mammary tissue accretion may remove approximately 35% of the protein that was available for maternal growth in early gestation (McPherson et al., 2004). The increase in BW gain observed in the current study could be attributed to increased fetal growth, or number born alive, however, there were no differences between control and L-Arg

supplemented gilts and sows for these 2 parameters. Thus, the improvement in late gestation BW gain observed in this study may be due to more closely meeting the increased nitrogen, or a specific arginine, requirement with supplementation in parity 0 and 1 gilts and sows that are still growing. Similar to other work, there were no differences in lactation BW loss (Mateo et al., 2008) or ADFI during lactation in pigs (Mateo et al., 2008) or rats (Zeng et al., 2008) between control and arginine supplemented groups when supplemental arginine was provided during gestation.

The majority of embryonic loss occurs before d 30 of gestation (Ford et al., 2002), with only a small percentage of losses occurring in the period of time in which supplementation of L-Arg was administered in the current study. In the current study, when L-Arg was supplemented from d 93 to 110 of gestation, there were no differences in number of pigs born alive, average birth weight, number of stillborns or mummies, or placental weight. Similarly, no improvement was observed in litter size or birth weight when sows were provided basal gestation diets supplemented with L-Arg from d 18 to 34 of gestation (Greiner et al., 2012; Zier-Rush et al., 2012), d 35 of gestation through farrowing (Zier-Rush et al., 2012), or d 75 to 115 of gestation (Greiner et al., 2012). However, when supplied from early gestation until parturition, supplementation of L-Arg increased total number and weight of piglets born alive and decreased number born dead per litter (Mateo et al., 2008). Additionally, feeding L-Arg supplemented diets from d 14 to 28 of gestation increased number of fetuses on d 70 of gestation (Bérard and Bee, 2010) and litter size in both primiparous and multiparous sows compared to controls (Ramaekers et al., 2006). Supplementation of control diets with 1.0% L-Arg from d 22 to d 114 of gestation increased litter size and total placental weight, with no difference in average birth weight, number of stillborns and mummies, or average placental weight (Gao et al., 2012). Pregnant rats

supplemented with 1.3% L-Arg had a 29% increase in implantation sites, and a 30% increase in litter size, compared to controls (Zeng et al., 2008). Finally, litter size and number of placental attachment sites increased, but average pup size decreased, when mice were provided diets supplemented with 2% L-Arg throughout gestation, compared to controls (Greene et al., 2012). Thus, the benefit observed in other studies may be due to improved early embryonic survival.

Supplementing basal gestation diets with 1.0% L-Arg during late gestation (d 93 to 110) did not have an effect on lactation (common diet) performance of the sow or her litter. In the current study there were no differences between L-Arg supplemented and control fed gilts and sows in number, or weight, of weaned pigs. Similarly, supplementation with 1.0% L-Arg from d 30 of gestation through parturition did not improve number weaned or weaning weight compared to control-fed animals (Easter and Baker, 1976; Mateo et al., 2008). However, piglets from sows fed a lactation diet supplemented with an additional 1.0% L-Arg had increased BW gain from d 0 to 7, and d 0 to 21, and were heavier at d 7, 14, and 21 of lactation compared to piglets of control fed sows (Mateo et al., 2008).

Arginine has been shown to stimulate the release of growth hormone and insulin in the pig (Cochard et al., 1998). Growth hormone stimulates the release of IGF-1 in a linear manner (Etherton et al., 1987). In the pig, circulating concentrations of plasma IGF-1 and insulin decrease from mating to parturition (Farmer et al., 2000). However, exogenous growth hormone increased circulating insulin concentration when administered from d 10 to 27 (Schneider et al., 2002) as well as the final 21 d (Kveragas et al., 1986) of gestation, likely due to insulin resistance. Additionally, exogenous growth hormone treatment from d 25 to 50 of gestation increased fetal weight on d 51 of gestation (Gatford et al., 2000), but had no effect on birth weight when administered the final 21 d of gestation (Kveragas et al., 1986). In the current

study circulating concentrations of plasma IGF-1 and insulin were not different on d 93 or d 110 of gestation between sows fed a control diet, or one supplemented with 1% L-Arg. Circulating levels of urea nitrogen may be used as an indicator of improved protein and amino acid efficiency, with lower concentrations of urea nitrogen indicative of improved use of dietary amino acids for protein accretion or fetal development. When fed diets supplemented with 1% L-Arg from d 30 of gestation, gilts had decreased plasma urea nitrogen on d 90 and 110 of gestation compared to controls (Mateo et al., 2007). In contrast, gestating sows provided 1% supplemental L-Arg from d 22 of gestation to d 114 had similar plasma urea nitrogen concentrations to isonitrogenous controls on d 90 of gestation (Gao et al., 2012). Similarly, sows in the current study did not differ in plasma urea nitrogen concentrations between arginine supplemented and the control diet fed sows on either day sampled.

In conclusion, supplementation of a control diet with an additional 1% arginine from d 93 to 110 of gestation increased BW gain in gilts and parity 1 sows. However, this increase in weight was independent of litter weight and had no effect on litter size at farrowing or subsequent lactation performance. Further research is warranted to determine the optimal time and level of arginine supplementation during gestation to achieve the greatest impact on litter and reproductive performance.

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Table 1. Composition of gestation and lactation diets[1]

Item	Experimental Diet, % (as-fed)		
	Gestation		Lactation
	CON	ARG	
Ingredient			
Corn	52.65	52.65	62.54
Soybean meal, 48%	9.00	9.00	21.25
Distillers dried grains with solubles	30.00	30.00	8.00
Fat (Yellow Grease)	1.00	1.00	2.75
Dicalcium phosphate	2.10	2.10	2.70
Limestone	1.15	1.15	0.70
Salt	0.45	0.45	0.40
Potassium magnesium sulfate[2]	0.65	0.65	0.50
Sow add pack[3]	0.25	0.25	0.25
Vitamin premix[4]	0.25	0.25	0.25
Mineral premix[5]	0.15	0.15	0.15
Ethoxyquin	0.03	0.03	0.03
Corn starch	—	1.05	—
L-Lysine	0.15	0.15	0.20
L-Threonine	—	—	0.03
L-Alanine	2.05	—	—
L-Arginine	—	1.00	—
Tylosin	0.125	0.125	—
Bacitracin methylene disalicylate	—	—	0.25

Table 1. (Cont.)

Calculated chemical composition[6]			
ME Mcal/kg	3.3	3.3	3.4
CP, %	18.7	18.7	17.6
SID Lys, %	0.63	0.63	0.90
SID M+C:Lys, %	82.9	82.9	58.45
SID Thr:Lys, %	74.4	74.4	63.34
SID Trp:Lys, %	19.2	19.2	18.45
SID Arg:Lys, %	119.5	276.4	108.0
Total P, %	0.83	0.83	0.88
Available P, %	0.58	0.58	0.58
Ca, %	0.99	0.99	0.98

[1]From d 93 to 110 gestation diets were provided at 2.72 kg/d. Lactation diets were provided ad libitum following farrowing. In order to make gestation diets isonitrogenous, 2.05% L-Ala was added to the control diet at the expense of corn starch, and 1.00% L-Arg was added to the ARG diets.

[2]Dynamate, Mosaic Feed Ingredients, Plymouth, MN

[3]The sow add pack provided the following per kg of complete diet: 22.05 IU of vitamin E, 551.15 mg of choline, 1.65 mg of folic acid, 4.96 mg of vitamin B_6, 0.22 mg of biotin, and 0.20 mg of chromium.

[4]The vitamin premix provided the following per kg of complete diet: 397.5 mg of Ca, 11,022.9 IU of vitamin A, 1,377.9 IU of vitamin D_3, 44.09 IU of vitamin E, 0.0386 mg vitamin B_{12}, 4.41 mg of menadione, 8.27 mg of riboflavin, 27.56 mg of D-pantothenic acid, and 49.6 mg of niacin.

[5]The mineral premix provided the following per kg of complete diet: 84 mg of Ca, 165 mg of Fe, 165 mg of Zn, 39.6 mg of Mn, 16.5 mg of Cu, 0.3 mg of I, and 0.3 mg of Se.

[6]ME – metabolizable energy; CP – crude protein; SID – standard ileal digestible;

Table 2. Sow and litter performance ($\bar{x}$ ± SEM)

Trait	Treatment		*P*-value
	Control	ARG[1]	
Farrowing BW change, kg	-21.22 ± 1.90	-22.37 ± 1.88	0.67
Lactation BW change, kg	-21.35 ± 1.82	-18.79 ± 1.86	0.33
Number born alive	13.59 ± 0.37	13.92 ± 0.37	0.53
Number weaned	10.65 ± 0.28	10.71 ± 0.28	0.89
Average piglet BW at birth, kg	1.41 ± 0.02	1.42 ± 0.02	0.69
Average piglet BW at weaning, kg	5.80 ± 0.13	5.89 ± 0.13	0.60
Number of stillborns	1.11 ± 0.22	1.05 ± 0.22	0.85
Number of mummies	0.52 ± 0.13	0.60 ± 0.13	0.67
Total BW of stillborns, kg	1.34 ± 0.28	1.29 ± 0.28	0.90
Total BW of mummies, kg	0.20 ± 0.08	0.21 ± 0.08	0.86
Placenta weight, kg	3.53 ± 0.16	3.70 ± 0.16	0.48

[1]ARG = 1.0% L-Arginine supplemented.

Table 3. Concentration of plasma IGF-1, insulin, and urea nitrogen ($\bar{x}$ ± SEM)

	Control		ARG[1]	
	Day 93	**Day 110**	**Day 93**	**Day 110**
IGF-1, ng/mL	48.96 ± 1.83	44.04 ± 2.02	47.54 ± 1.80	44.06 ± 2.01
Insulin, µU/mL	14.78 ± 1.09	17.61 ± 1.53	15.27 ± 1.08	15.24 ± 1.52
Urea nitrogen, mg/dL	4.15 ± 0.14	5.30 ± 0.17	3.98 ± 0.14	5.52 ± 0.17

[1]ARG = 1.0% L-Arginine supplemented.

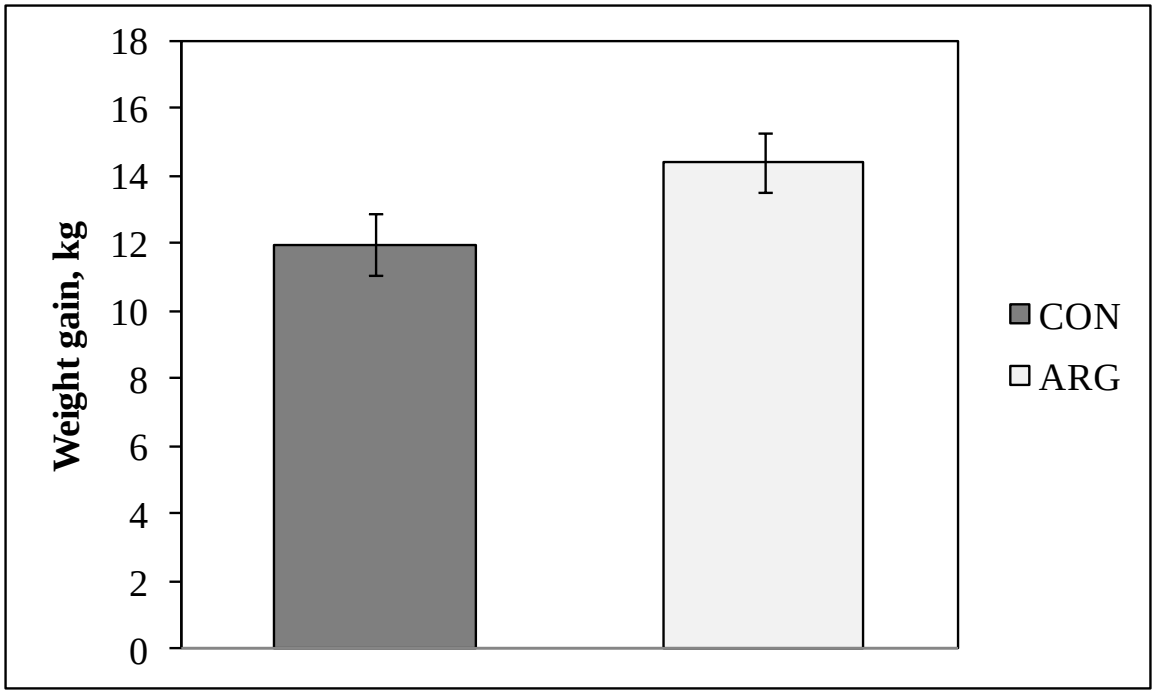

Figure 1. Late gestation (d 93 to d 110) body weight gain (*P* = 0.06). CON = Control, ARG = 1.0% L-Arginine supplemented.

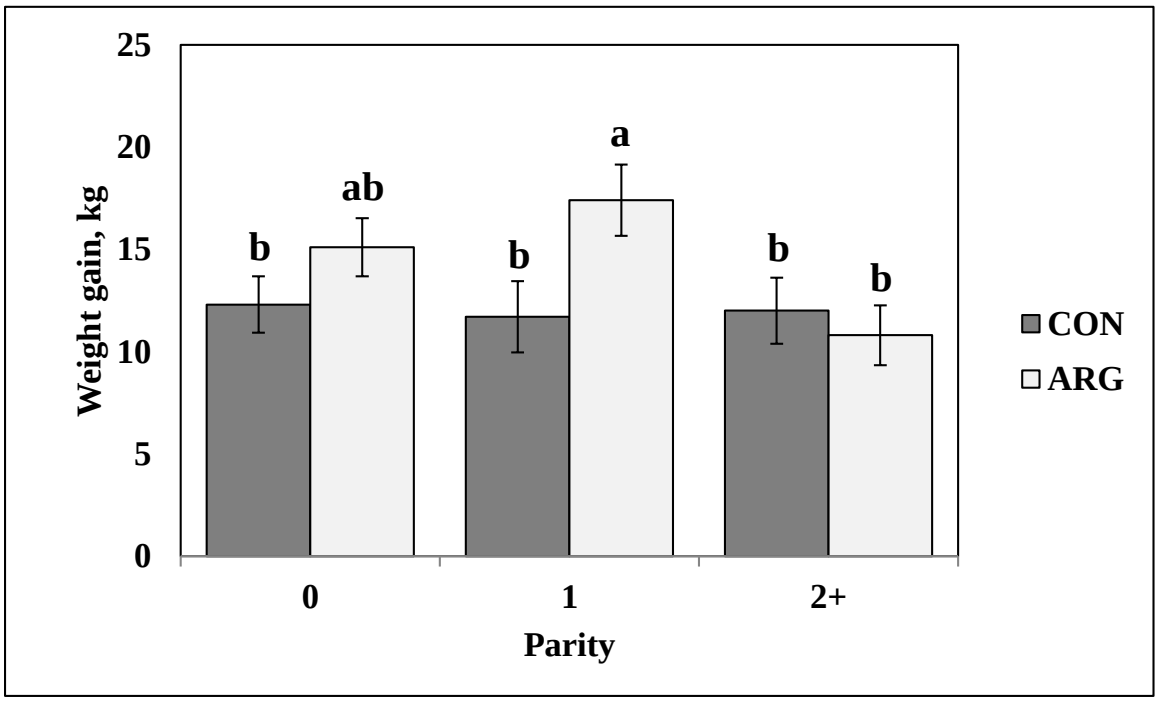

Figure 2. Gestation treatment x parity interaction (*P* = 0.1) for late gestation body weight gain. [ab] Bars with different letters differ ($P < 0.05$). CON = Control, ARG = 1.0% L-Arginine supplemented.

CHAPTER III
INFLUENCE OF A WHOLE YEAST PRODUCT FED THROUGHOUT GESTATION AND LACTATION ON PERFORMANCE AND IMMUNE PARAMETERS: I. SOW AND LITTER

Abstract

A study was conducted using 3 groups of gestating gilts and sows (n = 98) to determine the effects of *Pichia guilliermondii* (Pg), a whole yeast product (CitriStim; ADM Alliance Nutrition), on performance and immune parameters of dams and litters. Within 24 h of breeding, gilts and sows were allotted to 1 of 3 treatments consisting of a control (SC) diet or SC diet supplemented with 0.1 (S1) or 0.2% (S2) Pg. Dietary treatments were maintained through lactation. Colostrum and milk (d 14) samples were collected for IgA, IgG, and IgM analysis. Blood samples were collected from sows on d 110 of gestation for group 3, at weaning for all 3 groups, and from piglets at 14 d of age for white blood cell counts and serum IgA, IgG, and IgM analysis. Inclusion of Pg resulted in an increase in number born alive as the level of Pg increased (12.49, 13.33, and 13.43 born alive per litter for control, 0.1 and 0.2% Pg, respectively; linear effect, $P < 0.01$). Additionally, the percentage of piglets weighing less than 0.9 kg at birth was reduced in sows provided Pg at 0.1 or 0.2% compared with controls (linear effect, $P = 0.006$). Sows receiving Pg during gestation and lactation also weaned a greater number of piglets (10.31, 10.55, and 10.60 weaned per litter in control, 0.1 and 0.2% Pg, respectively; linear effect, $P < 0.01$) than controls. However, percent preweaning mortality was 17.58, 19.38, and 19.61% for control, 0.1 and 0.2% Pg, respectively (linear effect, $P < 0.02$). There were no differences in gestation BW gain, farrowing (d 110 to 48 h post-farrowing) or lactation BW loss (d 110 to weaning), individual birth and weaning weight, or number of mummies or stillborns. On d 110 of gestation the neutrophil concentration and neutrophil:lymphocyte ratio in peripheral blood were greater ($P < 0.02$) in S1 than SC, with S2 being intermediate. At weaning there was a liner

increase ($P < 0.03$) in neutrophil concentration, neutrophil:lymphocyte ratio, and percentage of neutrophils in the leukocyte population as level of Pg increased in sow diets. In conclusion, inclusion of Pg in sow diets increased, in a linear fashion, total number born alive and weaned, without a change in average birth or weaning weight, and decreased the number of lightweight pigs at birth. Additionally, inclusion of Pg had no effect on immune parameters measured in milk, colostrum, or d 14 piglet serum, but increased the peripheral blood neutrophil concentration of gilts and sows.

Introduction

Yeast products have been shown to enhance swine performance as both a probiotic (living, viable microorganism, such as active dry yeast) or prebiotic (nondigestible food ingredient, such as yeast cell culture). However, the effect of yeast products varies from no performance differences (Veum et al., 1995) to improvements in number born alive and weaned, as well as piglet body weight at birth, d 14 and 28 of lactation (Czech et al., 2010) or litter weaning weight and piglet average daily gain (Shen et al., 2011) in pigs from sows fed a yeast cell culture product.

Mannan oligosaccharides and β-glucans are components of the yeast cell wall that are believed to confer beneficial effects on immune parameters in sows (Shen et al., 2011). Several potential mechanisms have been proposed as to how these benefits are conveyed, including direct binding of pathogenic bacteria in the intestinal lumen (Spring et al., 2000), thereby flushing them from the digestive tract, as well as immunomodulation (Davis et al., 2004).

The majority of published studies involving yeast used supplements derived from *Saccharomyces cerevisiae*. Supplementation with yeast culture has been demonstrated to

improve milk production and feed intake in ruminants (Robinson and Garrett, 1999; Dann et al., 2000), as well as reproductive performance in pigs (Kim et al., 2008; Kim et al., 2010). Localized immunomodulatory effects in broilers in response to Pg, with no enhancement in performance, have been reported (Shanmugasundaram and Selvaraj, 2012). Therefore, the objective of this study was to determine the efficacy of CitriStim (ADM Alliance Nutrition), a whole yeast [*Pichia guilliermondii* (Pg)] coproduct of citric acid extraction, containing the whole yeast cell and its components, in enhancing dam and litter performance and modulating immune function.

Materials and Methods

Animals and Experimental Diets. Animal management and experimental procedures conducted during this study were approved by the University of Arkansas Institutional Animal Care and Use Committee.

A total of 98 GPK 35 gilts and sows were allotted to 1 of 3 dietary treatments based on parity, and body weight, at breeding. The 3 dietary treatments (Table 1) were a gestation control diet (SC), or the control diet supplemented with 0.1 (S1), or 0.2% Pg (S2). Gilts and sows were housed in individual gestation stalls (0.61 m x 2.13 m) and provided approximately 2.26 kg of feed per day and free access to water throughout the gestation period. On d 110 of gestation, gilts and sows were individually weighed and moved to the farrowing facility where they were housed in individual farrowing crates (1.22 m x 2.13 m). Upon farrowing, sows were fed ad libitum, maintaining gestation treatments through the lactation period. Both gestation and lactation diets were formulated to meet or exceed NRC (1998) requirements for gestating and

lactating sows, respectively (Table 1). Cross-fostering occurred within treatment groups and occurred within 24 h of farrowing.

Individual body weight was recorded for gilts and sows at breeding, 110 d of gestation, 48 h post farrowing, and at weaning, which occurred approximately 21 d post farrowing. Individual daily feed intake of each sow was also recorded during lactation. At farrowing, and again at weaning, the total number of live piglets was counted and individual piglet body weight was recorded. Additionally, the number of stillborns and mummies were recorded.

Within 24 h of parturition, colostrum samples were collected, and approximately 14 d later sows were administered oxytocin to stimulate milk letdown, and milk samples were collected to determine concentrations of IgA, IgG, and IgM.

Blood samples were obtained via jugular venipuncture from sows on d 110 (third farrowing group only), and at weaning (all 3 groups) and from 14 d old pigs to obtain a serum sample to determine concentrations of IgA, IgG, and IgM. An additional sample was obtained at each time point in tubes containing EDTA and analyzed for determination of peripheral blood white blood cell counts using a blood hematology system (Hemavet 950 FS, Drew Scientific, Waterbury, CT). A sample was also collected in EDTA tubes from 14 d old pigs for isolation of peripheral blood mononuclear cells for the interferon gamma induction assay; and in tubes containing sodium heparin to determine the phagocytic ability of phagocytes.

Chemical analysis. Colostrum, milk and serum samples were diluted and analyzed for IgA, IgG, and IgM with commercially available ELISA kits per manufacturer's instructions (Pig ELISA Quantitation Set, Bethyl Laboratories, Inc., Montgomery, TX).

Cytokine induction assay. The proliferative response of peripheral blood mononuclear cells was measured using an assay adapted from the methods of Blecha et al. (1983) and van

Heugten et al. (1994). Briefly, peripheral blood mononuclear cells were isolated by gradient centrifugation (Histopaque 1077, density = 1.077 g/mL; Sigma, St. Louis, MO). Aspirated cells were washed with RPMI 1640 (Sigma) supplemented with 1% antibiotic/antimycotic (Atlanta Biologicals, Norcross GA) and 5% fetal bovine serum (Premium; Atlanta Biologicals). Remaining erythrocytes were lysed by adding 1 mL of sterile water to the isolated cell pellet for 20 s, and isotonicity was restored by the addition of RPMI. Cells were pelleted and then resuspended in RPMI medium at 2×10^6 cells/mL and plated in triplicate in 96-well plates in 100 µl aliquots. Concanavalin A (ConA, Sigma) was administered in 50 µl aliquots to each well at a final concentration of 2.5, 5, and 10 µg/mL, to stimulate lymphocyte proliferation, whereas wells containing cells unstimulated with mitogen were administered 50 µl of medium only. Cells were incubated with ConA for 48 h at 39.2°C and 5% CO^2. At the completion of incubation, supernatant was transferred to a clean 96-well plate for analysis of interferon-gamma concentration using a commercially available ELISA kit, per manufacturer's instructions (Swine IFN-γ Antibody Pair, Invitrogen Corp., Camarillo, CA).

Phagocytosis assay. Phagocytic activity was determined in whole blood samples using a commercially available kit (pHrodo™ E. coli BioParticles® Phagocytosis Kit, Molecular Probes, Eugene, OR). Briefly, 20 µL pHrodo™ BioParticles® conjugate was added to tubes containing 100 µL whole blood sample and incubated for 15 min. Next, 100 µL Lysis Buffer A was added to all tubes, vortexed briefly, and incubated at room temperature for 5 min. Then, 1 mL Buffer B was added to each tube, vortexed briefly, and incubated at room temperature for 5 min. This was followed by centrifugation of samples at $350 \times g$ for 5 min at room temperature. Supernatant was discarded and the wash step was repeated with 1 mL Wash Buffer. Supernatant was again discarded and cells were resuspended in 0.5 mL Wash Buffer for flow cytometry analysis. A

FACSort flow cytometer and CellQuest software (Becton-Dickinson Immunocytometry Systems, San Jose, CA) were used to conduct analysis of the cell population. Two dot plots, one showing forward scatter (FSC) vs. side scatter (SSC), and the other showing FSC vs. fluorescence were created. A negative control sample (whole blood aliquot with no pHrodo™ BioParticles® conjugate that went through the preparation procedure) was applied to the flow cytometer, and linear FSC and SSC voltages were set to locate the leukocyte scatter pattern. For FSC vs. fluorescence, fluorescence was set on a log scale and events set in the lowest decade. Thresholds were then set to eliminate debris. A region was drawn around the granulocyte population and gated. Experimental samples were then applied and 10,000 events were recorded. Results were recorded as the percentage of phagocytosing cells and fluorescence intensity of the phagocytic cells.

***Statistical analysis*.** The PROC STEPWISE procedure of SAS was used to determine significant independent variables in the sow and litter study. Then, the PROC MIXED procedure of SAS was performed using relevant independent variables. Replicate and parity were included as random variables. Biological samples were analyzed using the PROC MIXED procedures of SAS with treatment as the fixed effect and parity as a covariate.

Results

There were no significant differences observed in gestation weight gain, total weight loss after farrowing, overall average daily feed intake, birth weight, number of stillborn or mummies, weaning weight or average daily gain. However, there was a tendency for increased average daily feed intake during week 3 in S1 gilts and sows (quadratic, $P = 0.07$), and a tendency for

decreased body weight loss in the same group for the 3-wk lactation period (quadratic, $P = 0.11$; Table 2).

Sows supplemented with Pg had almost 1 pig per litter more than those receiving the control diet ($P = 0.004$; Figure 1), however average piglet birth weight did not differ. Thus, pig size was consistent though litter size increased in sows provided diets supplemented with Pg. Additionally, sows provided Pg had a lower percentage of pigs weighing less than 0.91 kg at birth ($P = 0.02$; Figure 2). Sows provided Pg during gestation and lactation had a small, but significant increase in the number of pigs weaned ($P = 0.02$; Figure 3). It should be noted that, similar to the increase in number born alive, there was no difference among groups in average weaning weight. However, there was a small increase in preweaning mortality for sows provided Pg ($P = 0.03$; Figure 4).

No differences were observed in IgA, IgG, or IgM among treatments in colostrum, milk, or serum from sows or 14 d old piglets (Tables 3, 4, and 5). However, the neutrophil concentration and the neutrophil:lymphocyte ratio were greater on d 110 of gestation in S1 than SC or S2 (quadratic, $P < 0.05$; Table 6). Additionally, lymphocytes comprised a greater ($P = 0.03$) percentage of leukocytes in SC and S2 than S1 (quadratic, $P = 0.03$). At weaning, a liner increase ($P < 0.03$) in neutrophil concentration, neutrophil:lymphocyte ratio (1.66, 1.96, and 2.34 for SC, S1, and S2, respectively), and percentage of neutrophils in the leukocyte population was observed as Pg increased in sow diets (Table 6). No difference was observed in the concentration and proportions among peripheral blood leukocytes of 14 d old suckling pigs (Table 7).

There were no differences in the percentage of phagocytic monocytes or granulocytes in the whole blood preparation, nor was there a difference in fluorescence intensity of the

phagocytic granulocytes (Table 8). However, a linear decrease was observed in fluorescence intensity of phagocytic monocytes as the level of Pg increased in sow diets ($P = 0.02$). Additionally, ConA stimulated interferon-gamma production was greater in peripheral blood mononuclear cells isolated from the blood of 14 d old suckling pigs from sows receiving diets supplemented with increasing levels of Pg (linear, $P = 0.03$; Table 9).

Discussion

Yeast culture is a dried yeast fermentation product containing yeast, as well as metabolites from the culture media in which it was grown. Many yeast culture products are derived from *Saccharomyces cerevisiae*; however, other yeast products exist that have not been as extensively studied. Data from the current study indicate a potential benefit to dietary supplementation during gestation and lactation with a product derived from *Pichia guilliermondii*.

Supplementing gestation diets with 0.1 or 0.2% Pg did not improve gestation body weight gain when compared to animals receiving control diets. No difference in gestation body weight gain was observed when yeast culture was supplemented throughout gestation (Shen et al., 2011), or beginning at d 60 of gestation (Veum et al., 1995). In both of these studies dietary treatment was carried through lactation and no difference was observed in lactation body weight loss as well. In the current study there was a tendency (quadratic, $P = 0.11$) for lactation body weight loss to be decreased in sows receiving 0.1% Pg compared to those given the control or 0.2% Pg diet.

Daily feed intake was similar among dietary treatment groups during the first 2 weeks of lactation. However, during week 3 there was a tendency for increased ADFI in sows that

received lactation diets supplemented with 0.1% Pg (quadratic, $P = 0.07$). Others have reported no difference in ADFI between sows fed control diets or yeast culture supplemented diets over the lactation period (Veum et al., 1995; Kim et al., 2008; and Shen et al., 2011) or even an increase in ADFI for primiparous gilts fed control diets compared to those fed yeast culture supplemented diets (Kim et al., 2010).

Several previous studies have reported no improvement in litter size at birth with yeast culture supplementation during gestation (Veum et al., 1995; Kim et al., 2008; and Shen et al. 2011). Czech and associates (2010) reported that sows provided yeast culture supplementation beginning at 4 wk prior to farrowing had increased number born alive, increased body weight at birth, and decreased number of stillborns compared to sows provided a control diet. Similarly, we found that supplementation with Pg throughout gestation increased number born alive by almost 1 pig per litter, and decreased the percentage of lightweight (≤ 0.91 kg) pigs at birth ($P = 0.02$) with increasing dietary inclusion of Pg. Additionally, the number of pigs per litter at weaning was improved in sows that received diets supplemented with Pg during gestation and lactation compared to those that received control diets. The percentage of preweaning mortality was greater for sows provided Pg supplemented compared to the control diet. It should be noted that at least 50% of mortalities were caused by pigs being crushed by the sow. However, no differences were observed between sows provided the control diet or Pg supplemented diets in total birth weight, number of stillborns, total or individual weaning weight, or average daily gain. Others have reported improvements in number weaned (Czech et al., 2010), and litter weight gain (Kim et al., 2008; and Shen et al., 2011) in sows provided yeast culture. These authors attributed the improvement at weaning to potential improvements in milk production, milk quality, nutrient digestibility, or immune status provided by yeast culture supplementation. In

the current study, increased feed intake in wk 3 of lactation did not improve litter weight at weaning, or average daily gain of nursing piglets.

To further examine how Pg affected sow and litter immune parameters we measured peripheral blood white blood cell counts and serum immunoglobulin concentrations from sows on d 110 of gestation and at weaning, as well as suckling pigs at approximately d 14 of age. Additionally, immunoglobulin concentrations of colostrum and milk were examined. Supplementation of gestation diets with Pg did not change serum concentrations of IgA, IgG, or IgM on d 110 of gestation, or at weaning, when compared to sows fed the control diet. Similarly, concentrations of IgA, IgG, and IgM in colostrum, nor milk, were not different among treatment groups. Based on these data, as expected, there were also no differences among treatments in serum concentrations of IgA, IgG, or IgM of 14 d old suckling pigs. Shen and associates (2011) also reported no difference in IgG concentration of colostrum or milk of the sow, or serum of 17 d old suckling pigs, when sows were provided control or yeast culture supplemented gestation and lactation diets. In contrast, Czech et al. (2010) observed that sows provided yeast culture from d 84 of gestation through a 28 d lactation period had increased serum IgG on d 110 of gestation and IgA on d 21 of lactation. Additionally, colostrum concentrations of IgG and IgM were increased for sows that were fed diets supplemented with yeast culture compared to those provided control diets. Piglets from these sows had increased serum concentrations of IgG and IgM at birth, and IgG concentrations remained elevated at d 21 of age. Factors such as diet and immune challenge can affect immunoglobulin levels in serum and colostrum. Nonspecific immunostimulation of sows 4 to 6 weeks prior to parturition caused an increase in serum, as well as colostral, IgG concentration and improved body weight of piglets suckling sows that had been immunostimulated at 2 and 28 d of age compared to control sows

(Krakowski et al., 2002). Thus, stimulation of the immune system near parturition may cause the improvement observed in colostrum and piglet performance.

Sows that were provided a gestation diet supplemented with 0.1% Pg tended to have more leukocytes in the peripheral circulation than those provided the control diet or diet supplemented with 0.2% Pg. Additionally, peripheral blood of sows that received the gestation diet supplemented with 0.1% Pg had a greater neutrophil concentration, a greater percentage of neutrophils in the leukocyte population, and decreased percentage of lymphocytes in the leukocyte population, which increased the neutrophil:lymphocyte ratio. At weaning, a linear increase in neutrophils, the percentage of leukocytes that were neutrophils, and neutrophil:lymphocyte ratio was observed as the level of Pg increased in gestation and lactation diets. These data are contrary to that of Shen et al. (2011), who reported a decrease in peripheral blood neutrophil concentration in response to supplementation of a *Saccharomyces cerevisiae* fermentation product. It is interesting to note that these differences were not apparent on d 30 of gestation in the study by Shen, indicating there may be a conditioning period needed to see the effects of the yeast culture product supplementation in blood cell counts. The increase in peripheral blood neutrophil concentration in the current study may indicate that sows provided Pg had an increased inflammatory challenge compared to those receiving the control diet as neutrophils represent the first line of defense in an infection. Additionally, increased neutrophil:lymphocyte ratio is associated with stress in pigs (Stull et al., 1999) and a reduction in neutrophils with concurrent increase in lymphocytes was determined to be an alleviation of inflammation in weaned pigs (Davis et al., 2004), though the opposite was observed in the current study. Unlike their dams, there were no differences in the proportion among leukocytes observed in 14 d old suckling pigs in the current study. This is in agreement with Shen, who

found modulations in immune cell numbers in sows fed a yeast culture product compared to those receiving a control diet, but did not observe differences in the proportion among leukocytes in 17 d old suckling pigs.

In 14 d old pigs, in the current study, there was no difference in the percentage of monocytes in whole blood that were phagocytically active, however the fluorescence intensity decreased linearly as the level of Pg in sow diets increased. This may indicate that addition of Pg in sow diets decreased the number of particles that were phagocytosed when compared to controls. Supplementation of sow diets with 0.2% Pg linearly increased production of interferon-gamma from peripheral blood mononuclear cells in response to concanavalin A, a T-cell mitogen that stimulates interferon-gamma production. This would indicate that a greater lymphocyte proliferation occurred in pigs from sows that were fed 0.2% Pg during gestation and lactation compared to those receiving 0.1% Pg and controls. Interestingly, others have reported that mannans, which are present in the walls of yeast cells, may inhibit lymphocyte function (Muchmore et al., 1990; Podzorzki et al., 1990), but was not apparent in the current study. Although the amount of Pg the suckling piglets ingested in this study is unknown.

In summary, supplementation of standard gestation and lactation diets with Pg may be beneficial to sow reproductive performance, as well as the performance of her litter as manifested in an improvement in litter size at birth, as well as at weaning, and a reduction in light weight pigs at birth. Additionally, it appears that supplementation of Pg to gestation and lactation diets alters the immune profile of sow serum, but not colostrum or milk, when provided throughout gestation and lactation. Thus, *Pichia guilliermondii* may be a viable alternative to other yeast culture products in its ability to improve performance and act as an immunomodulator.

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Table 1. Composition (as-fed) of gestation and lactation diets[1].

Ingredients (%)	Gestation	Lactation
Corn	54.52	54.81
Soybean meal, 48%	9.50	28.00
Dried distillers grains with solubles	30.00	10.00
Fat (yellow grease)	1.00	2.00
Dicalcium phosphate	1.875	2.40
Limestone	1.175	0.75
Salt	0.45	0.50
L-Lysine	0.15	0.175
L-Threonine	0.00	0.04
Potassium magnesium sulfate[2]	0.65	0.65
Sow add pack[3]	0.25	0.25
Vitamin premix[4]	0.25	0.25
Mineral premix[5]	0.15	0.15
Ethoxyquin	0.03	0.03
Calculated composition[6]		
ME (Mcal/kg)	1.50	1.51
CP (%)	17.07	20.67
SID Lysine (%)	0.652	1.065
Available P (%)	0.544	0.547
Ca (%)	0.952	0.956
SID M+C:Lys	81	56
SID Thr:Lys	73	64
SID Trp:Lys	19	19
SID Ile:Lys	80	69
SID Val:Lys	100	78

[1]Control diets for gestation and lactation. For the S1 and S2 diets Pg was added at the expense of corn.

[2]Dynamate, Mosaic Feed Ingredients

[3]The sow add pack provided the following per kg of complete diet: 22.05 IU of vitamin E, 551.15 mg of choline, 1.65 mg of folic acid, 4.96 mg of vitamin B_6, 0.22 mg of biotin, and 0.20 mg of chromium.

[4]The vitamin premix provided the following per kg of complete diet: 397.5 mg of Ca, 11,022.9 IU of vitamin A, 1,377.9 IU of vitamin D_3, 44.09 IU of vitamin E, 0.0386 mg vitamin B_{12}, 4.41 mg of menadione, 8.27 mg of riboflavin, 27.56 mg of D-pantothenic acid, and 49.6 mg of niacin.

[5]The mineral premix provided the following per kg of complete diet: 84 mg of Ca, 165 mg of Fe, 165 mg of Zn, 39.6 mg of Mn, 16.5 mg of Cu, 0.3 mg of I, and 0.3 mg of Se.

[6]ME – metabolizable energy; CP – crude protein; SID – standard ileal digestible.

Table 2. Effect of supplementation of *Pichia guilliermondii* on sow reproductive performance.

Variable[1]	SC[2]	S1	S2	*P*-value	Linear *P*-value	Quadratic *P*-value
Sow gestation BW gain, kg	51.26 ± 2.53	53.82 ± 2.51	52.50 ± 2.52	0.42	0.52	0.24
Sow farrowing BW loss, kg	-11.56 ± 2.53	-14.12 ± 2.51	-12.81 ± 2.52	0.42	0.52	0.24
Sow lactation BW loss, kg	-12.50 ± 4.62	-7.89 ± 4.59	-10.05 ± 4.61	0.17	0.32	0.11
Sow total BW loss, kg	-23.85 ± 2.92	-23.34 ± 2.88	-22.53 ± 2.88	0.88	0.62	0.95
Week 1 lactation ADFI, kg	4.64 ± 0.20	4.51 ± 0.20	4.63 ± 0.20	0.74	0.95	0.44
Week 2 lactation ADFI, kg	7.00 ± 0.15	6.87 ± 0.15	6.96 ± 0.14	0.80	0.84	0.52
Week 3 lactation ADFI, kg	7.19 ± 0.15	7.53 ± 0.14	7.26 ± 0.14	0.19	0.73	0.07
Overall lactation ADFI, kg	6.17 ± 0.39	6.39 ± 0.39	6.24 ± 0.39	0.68	0.78	0.40
Total birth weight, kg	17.73 ± 0.55	16.97 ± 0.53	17.42 ± 0.53	0.57	0.67	0.32
Average birth weight, kg	1.35 ± 0.01	1.34 ± 0.01	1.34 ± 0.01	0.79	0.52	0.80
Number of stillborn	0.86 ± 0.22	0.82 ± 0.21	1.17 ± .021	0.38	0.28	0.42
Number of mummies	0.44 ± 0.11	0.22 ± 0.11	0.39 ± 0.11	0.32	0.73	0.14
Total weaning weight, kg	63.14 ± 1.65	61.95 ± 1.61	61.65 ± 1.60	0.75	0.47	0.80
Average weaning weight, kg	6.03 ± 0.16	5.91 ± 0.15	5.83 ± 0.15	0.62	0.33	0.90
Piglet average daily gain, kg/d	0.22 ± 0.002	0.22 ± 0.001	0.22 ± 0.001	0.38	0.24	0.45
Percent weaned ≤ 3.18 kg	3.60 ± 1.33	5.11 ± 1.31	6.28 ± 1.31	0.37	0.16	0.91

[1]BW – body weight; ADFI – average daily feed intake.

[2]SC – Sow control diet; S1- Control diet supplemented with 0.1% Pg; S2 – Control diet supplemented with 0.2% Pg.

Table 3. Effects of *Pichia guilliermondii* (Pg) on sow serum IgA, IgG, and IgM concentration at gestation d 110 and weaning.

Item	SC[1]	S1	S2	*P*-value	Linear *P*-value	Quadratic *P*-value
IgA, mg/mL[2]						
d 110	0.89 ± 0.16	1.03 ± 0.16	0.80 ± 0.18	0.62	0.71	0.36
Weaning	1.04 ± 0.11	1.19 ± 0.11	1.20 ± 0.11	0.51	0.30	0.59
IgG, mg/mL						
d 110	15.13 ± 2.42	13.62 ± 2.42	13.03 ± 2.55	0.57	0.32	0.79
Weaning	15.39 ± 2.08	15.50 ± 2.07	14.94 ± 2.07	0.86	0.67	0.72
IgM, mg/mL[2]						
d 110	4.83 ± 0.65	6.19 ± 0.65	5.24 ± 0.74	0.34	0.68	0.17
Weaning	2.87 ± 0.22	3.29 ± 0.21	3.08 ± 0.21	0.39	0.48	0.23

[1]SC – Sow control diet; S1- Control diet supplemented with 0.1% Pg; S2 – Control diet supplemented with 0.2% Pg.

[2]Gestation d 110 differs from weaning ($P < 0.001$).

Table 4. Effect of *Pichia guilliermondii* (Pg) on IgA, IgG, and IgM concentration of sow colostrum and milk[1].

Item	SC[1]	S1	S2	*P*-value	Linear *P*-value	Quadratic *P*-value
IgA, mg/mL[3]						
Colostrum	10.06 ± 0.92	11.24 ± 0.91	10.27 ± 0.92	0.37	0.81	0.17
Milk	5.14 ± 0.43	5.34 ± 0.43	5.05 ± 0.43	0.71	0.80	0.43
IgG, mg/mL[3]						
Colostrum	65.65 ± 5.46	68.47 ± 5.42	70.23 ± 5.48	0.59	0.31	0.89
Milk	0.38 ± 0.05	0.38 ± 0.05	0.37 ± 0.05	0.92	0.91	0.70
IgM, mg/mL[3]						
Colostrum	5.50 ± 0.47	5.71 ± 0.47	5.51 ± 0.48	0.90	0.98	0.65
Milk	2.43 ± 0.29	2.54 ± 0.28	2.63 ± 0.29	0.72	0.42	0.95

[1]Colostrum collected following farrowing. Milk collected at approximately d 14 postfarrowing.
[2]SC – Sow control diet; S1- Control diet supplemented with 0.1% Pg; S2 – Control diet supplemented with 0.2% Pg.
[3]Colostrum differs from milk ($P < 0.0001$).

Table 5. Effect of *Pichia guilliermondii* (Pg) fed to sows on nursing piglet serum IgA, IgG, and IgM concentration on d 14.

Item	SC[1]	S1	S2	*P*-value
IgA, mg/mL	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.95
IgG, mg/mL	9.27 ± 1.20	9.15 ± 1.19	9.78 ± 1.20	0.82
IgM, mg/mL	4.11 ± 0.70	4.46 ± 0.70	4.90 ± 0.70	0.31

[1]SC – Sow control diet; S1- Control diet supplemented with 0.1% Pg; S2 – Control diet supplemented with 0.2% Pg.

Table 6. Effect of *Pichia guilliermondii* (Pg) on concentration and proportions among peripheral blood leukocytes of sows at gestation d 110 and weaning.

	SC[1]	S1	S2	*P*-value	Linear *P*-value	Quadratic *P*-value
d 110 Gestation						
WBC (K/uL)[2]	9.99 ± 0.62	11.76 ± 0.66	10.58 ± 0.73	0.17	0.54	0.09
NE (K/uL)	4.17 ± 0.49[b]	6.44 ± 0.52[a]	5.60 ± 0.58[ab]	0.02	0.07	0.03
LY (K/uL)	3.31 ± 0.24	2.82 ± 0.26	2.91 ± 0.29	0.36	0.30	0.39
MO (K/uL)	0.22 ± 0.02	0.22 ± 0.03	0.21 ± 0.03	0.95	0.76	0.89
EO (K/uL)	2.15 ± 0.29	2.12 ± 0.21	1.78 ± 0.35	0.69	0.42	0.70
BA (K/uL)	0.14 ± 0.03	0.15 ± 0.03	0.07 ± 0.04	0.32	0.20	0.35
NE:LY	1.30 ± 0.31[b]	2.59 ± 0.33[a]	2.12 ± 0.37[ab]	0.03	0.10	0.04
NE (%)	42.54 ± 3.61[b]	54.79 ± 3.86[a]	52.32 ± 4.26[ab]	0.07	0.10	0.14
LY (%)	33.16 ± 1.98[a]	24.60 ± 2.11[b]	27.89 ± 2.34[ab]	0.03	0.10	0.03
MO (%)	2.23 ± 0.22	1.88 ± 0.23	2.05 ± 0.26	0.57	0.61	0.38
EO (%)	20.74 ± 2.26	17.48 ± 2.41	17.00 ± 2.68	0.49	0.30	0.65
BA (%)	1.33 ± 0.28	1.24 ± 0.30	0.73 ± 0.33	0.37	0.18	0.57
Weaning						
WBC (K/uL)	12.21 ± 0.65	12.25 ± 0.64	12.39 ± 0.62	0.98	0.84	0.95
NE (K/uL)	5.07 ± 0.39	5.76 ± 0.38	6.28 ± 0.37	0.82	0.03	0.85
LY (K/uL)	3.19 ± 0.15	2.99 ± 0.14	2.94 ± 0.14	0.45	0.23	0.68
MO (K/uL)	0.33 ± 0.03	0.39 ± 0.03	0.33 ± 0.03	0.21	0.87	0.08
EO (K/uL)	3.32 ± 0.37	2.88 ± 0.36	2.64 ± 0.35	0.39	0.17	0.82
BA (K/uL)	0.30 ± 0.06	0.21 ± 0.06	0.19 ± 0.06	0.36	0.17	0.64
NE:LY	1.66 ± 0.19[b]	1.96 ± 0.18[ab]	2.34 ± 0.18[a]	0.03	0.01	0.86
NE (%)	43.11 ± 2.05[b]	47.04 ± 2.00[ab]	50.08 ± 1.95[a]	0.05	0.01	0.86
LY (%)	26.84 ± 1.14	25.11 ± 1.12	24.62 ± 1.09	0.33	0.16	0.65
MO (%)	2.66 ± 0.22	3.20 ± 0.21	2.76 ± 0.21	0.17	0.72	0.06
EO (%)	25.32 ± 1.73	22.97 ± 1.69	21.02 ± 1.64	0.19	0.07	0.92
BA (%)	2.07 ± 0.26	1.67 ± 0.25	1.52 ± 0.24	0.27	0.11	0.69

[1]SC – Sow control diet; S1- Control diet supplemented with 0.1% Pg; S2 – Control diet supplemented with 0.2% Pg.

[2]WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/µL) – 1,000 cells/µL; (%) - percentage of total WBC.

[ab]Means in the same row with different superscripts are different ($P < 0.05$).

Table 7. Effect of *Pichia guilliermondii* (Pg) on concentration and proportions among peripheral blood leukocytes of piglets at 14 d of age.

Item[2]	SC[1]	S1	S2	*P*-value	Linear *P*-value	Quadratic *P*-value
Age	13.26 ± 1.06	13.56 ± 1.19	13.44 ± 1.25			
WBC (K/uL)	8.98 ± 0.60	9.38 ± 0.59	9.54 ± 0.60	0.78	0.50	0.87
NE (K/uL)	3.25 ± 0.29	3.34 ± 0.29	3.41 ± 0.29	0.92	0.69	0.97
LY (K/uL)	4.57 ± 0.36	4.86 ± 0.36	5.06 ± 0.37	0.61	0.33	0.92
MO (K/uL)	0.49 ± 0.04	0.54 ± 0.04	0.49 ± 0.04	0.68	0.95	0.38
EO (K/uL)	0.62 ± 0.08	0.59 ± 0.08	0.52 ± 0.08	0.63	0.35	0.88
BA (K/uL)	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.88	0.61	0.96
NE:LY	0.82 ± 0.09	0.72 ± 0.09	0.77 ± 0.09	0.71	0.69	0.47
NE (%)	34.89 ± 1.87	35.71 ± 1.86	36.46 ± 1.90	0.83	0.55	0.99
LY (%)	51.84 ± 1.92	51.48 ± 1.91	52.44 ± 1.95	0.94	0.82	0.78
MO (%)	5.79 ± 0.44	5.76 ± 0.44	5.43 ± 0.44	0.81	0.56	0.78
EO (%)	7.02 ± 0.77	6.52 ± 0.76	5.11 ± 0.77	0.18	0.08	0.62
BA (%)	0.46 ± 0.10	0.52 ± 0.10	0.56 ± 0.10	0.72	0.43	0.91

[1]SC – Sow control diet; S1- Control diet supplemented with 0.1% Pg; S2 – Control diet supplemented with 0.2% Pg.

[2]WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/µL) – 1,000 cells/µL; (%) - percentage of total WBC.

Table 8. Effect of *Pichia guilliermondii* (Pg) on monocyte and granulocyte phagocytosis of 14 d old suckling piglets.

				P-value				
	SC[1]	S1	S2	age	sex	trt	linear	quadratic
Monocytes								
% of total	76.31	73.34	73.20	0.30	0.03	0.74	0.49	0.71
% phagocytically active	5.96	5.27	5.64	0.03	0.16	0.62	0.65	0.39
Fluorescence intensity	84.24[a]	80.18[ab]	78.26[b]	0.86	0.68	0.05	0.02	0.62
Granulocytes								
% of total	87.33	85.55	84.1	0.96	0.24	0.63	0.34	0.96
% phagocytically active	15.34	13.87	13.25	0.72	0.78	0.32	0.14	0.72
Fluorescence intensity	187.73	192.66	197.67	0.77	0.89	0.79	0.49	1.00

[1]SC – Sow control diet; S1- Control diet supplemented with 0.1% Pg; S2 – Control diet supplemented with 0.2% Pg.

[ab]Means in the same row with different superscripts are different ($P < 0.05$).

Table 9. Effect of *Pichia guilliermondii* (Pg) on the response of PBMC of 14 d old suckling piglets to stimulation with ConA.[1]

	IFN-gamma (pg/mL)
ConA (ug/mL); $P = 0.0021$	
0	42.14[b]
2.5	55.50[b]
5	203.73[b]
10	542.16[a]
Sow Treatment; $P = 0.0419$	
SC[2]	118.94[b]
S1	119.38[b]
S2	394.32[a]
Sow Treatment at 10 ug/mL ConA	
SC	273.16 ± 261.95[b]
S1	302.50 ± 246.34[b]
S2	1050.82 ± 267.44[a]
P-value	
Age	0.48
Sex	0.29
ConA linear	0.0002
Sow diet linear	0.03
Sow diet quadratic	0.19

[1]PBMC – peripheral blood mononuclear cells; ConA – concanavalin A.

[2]SC – Sow control diet; S1- Control diet supplemented with 0.1% Pg; S2 – Control diet supplemented with 0.2% Pg.

[ab]Means in the same column (within main effect) with different superscripts are different ($P < 0.05$).

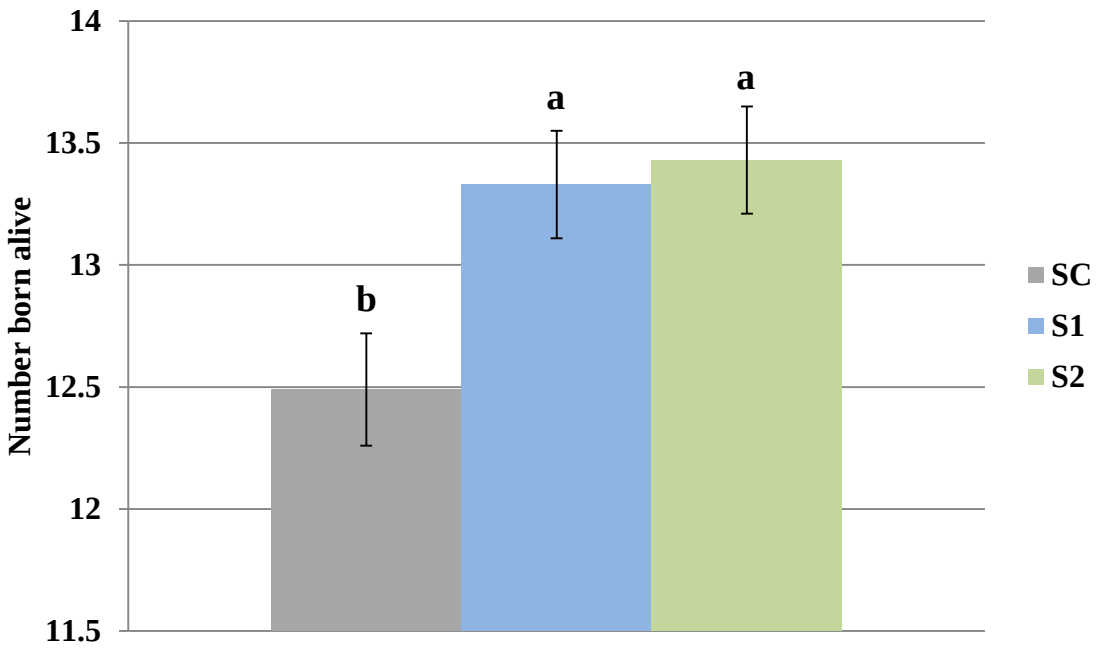

Figure 1. Effect of *Pichia guilliermondii* (Pg) on number of piglets born alive (*P* = 0.004). SC – Sow control diet; S1 – Control + 0.1% Pg; S2 – Control + 0.2% Pg. [ab]Bars with different letters differ ($P < 0.05$).

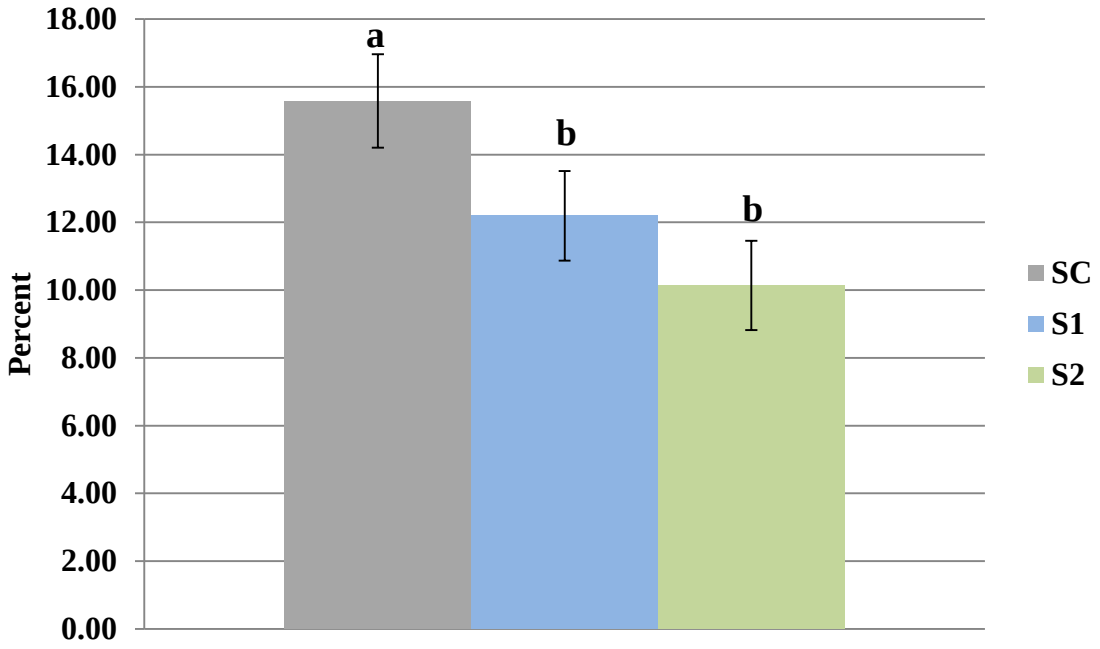

Figure 2. Effect of *Pichia guilliermondii* (Pg) on percentage of piglets born alive weighing less than, or equal to, 0.91 kg (*P* = 0.02). SC – Sow control diet; S1 – Control + 0.1% Pg; S2 – Control + 0.2% Pg. [ab]Bars with different letters differ ($P < 0.05$).

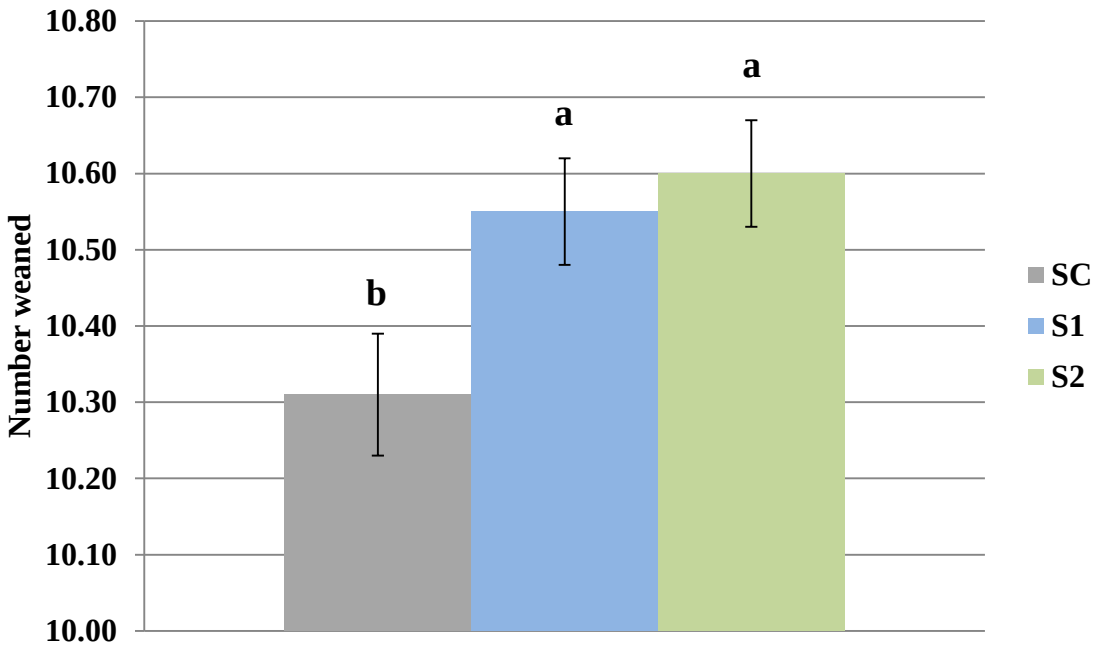

Figure 3. Effect of *Pichia guilliermondii* (Pg) on total number of pigs weaned (*P* = 0.02). SC – Sow control diet; S1 – Control + 0.1% Pg; S2 – Control + 0.2% Pg. [ab]Bars with different letters differ ($P < 0.05$).

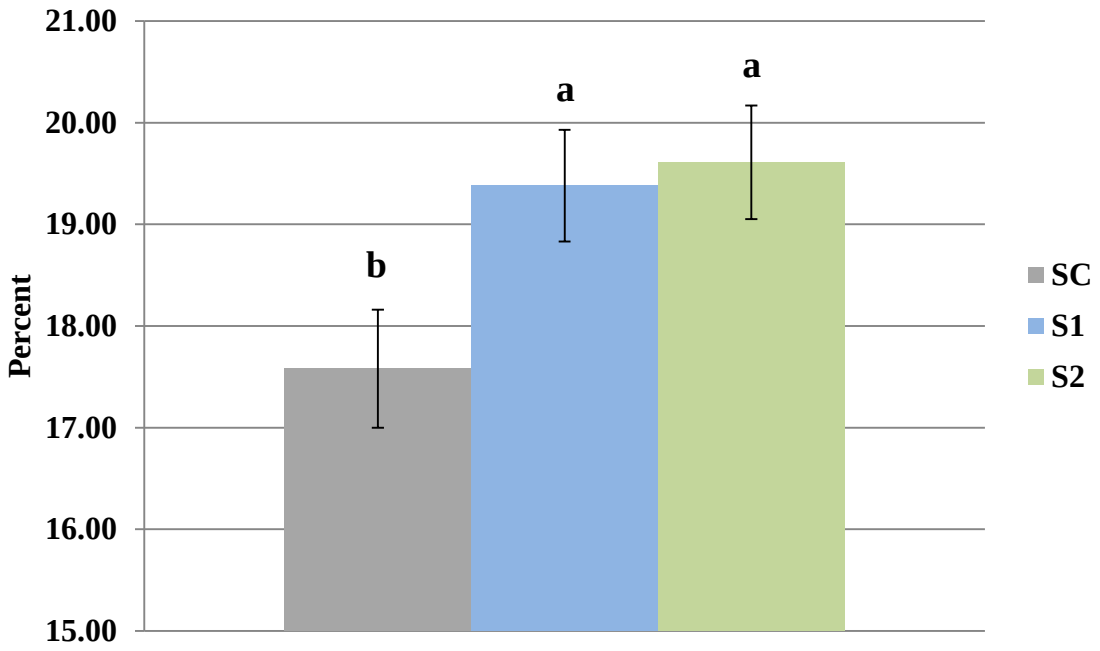

Figure 4. Effect of *Pichia guilliermondii* (Pg) on percentage preweaning mortality (*P* = 0.03). SC – Sow control diet; S1 – Control + 0.1% Pg; S2 – Control + 0.2% Pg. [ab]Bars with different letters differ ($P < 0.05$).

CHAPTER IV
INFLUENCE OF A WHOLE YEAST PRODUCT FED THROUGHOUT GESTATION AND LACTATION ON PERFORMANCE AND IMMUNE PARAMETERS: II. THE WEANED PIG

Abstract

To determine the impact on performance and immune parameters of progeny from sows fed a whole yeast product (*Pichia* guilliermondii; Pg) throughout gestation and lactation, pigs from 2 separate farrowing groups were weaned (21 d) and allotted in a 3 (sows fed 0 [SC], 0.1 [S1], or 0.2% [S2] Pg) x 2 (nursery with [NPg] or without [NC] Pg) factorial arrangement in an randomized complete block design (n = 336 and 288 pigs in Exp. 1 and 2, respectively). Pigs were provided feed and water ad libitum during phase 1, 2, and 3 for 7, 14, and 14 d, respectively, in both studies. In Exp. 1, blood samples were collected on d 5 and 28 post-weaning for analysis. During Exp. 2 (Exp. 2A), 36 pigs were individually penned and challenged with lipopolysaccharide (25 µg/kg BW i.m.; 0 h). Individual BW, ADFI, and rectal temperature were collected at -48, -24, 0, 24, and 48 h, and blood samples at 0, 5, and 24 h for analysis. In Exp. 1, Pg inclusion in sow diets increased ADG during the overall phase 1 and 2 period, and BW at the end of phase 2 ($P = 0.02$). Additionally, ADG, ADFI, and BW increased in phase 1 as level of Pg in sow diet increased in weaned pigs fed the diet supplemented with Pg, with no change in pigs provided the nursery control diet regardless of sow treatment (interaction; $P < 0.05$). In the combined phase 1 and 2 periods, phase 3, and the overall nursery period ADFI increased as level of Pg increased in sow diets in NPg, whereas a decrease was observed as sow level of Pg increased in NC weaned pigs (interaction; $P < 0.04$). An increase in G:F was observed in phase 2, the combined phase 1 and 2 period, and the overall nursery period in NC weaned pigs as level of Pg increased in sow diets, however NPg had similar G:F regardless of sow treatment (interaction; $P < 0.03$). Pigs from S1 had increased overall serum IgG (sow

quadratic [Q], $P < 0.05$). The percentage of leukocytes that were eosinophils in peripheral blood was greater in pigs from S2 fed NPg on d 5 than NC (sow linear x nursery, $P < 0.05$). On d 28, leukocyte and eosinophil concentrations were greater in pigs from SC (Q, $P < 0.05$). In Exp. 2, a linear increase ($P < 0.02$) was observed in ADG, ADFI and G:F for phase 1, phase 2, the combined phase 1 and 2 period, and the overall nursery period, and BW at the end of phase 2 and phase 3 increased linearly ($P < 0.01$) with increasing Pg in sow diets. For Exp. 2A, a linear increase ($P < 0.05$) in ADFI was observed at -24 to 0 h, -48 to 0 h, and 24 to 48 h, and in rectal temperature at -48, -24, 0 and 5 h with increasing Pg inclusion in sow diets. However, rectal temperature decreased more from 5 h to 24 h in pigs from sows receiving Pg. The peripheral blood concentration of monocytes and percentage of monocytes in the leukocyte population were greater in S2 compared to SC and S1 ($P < 0.05$). Also, the peripheral blood neutrophil concentration was reduced at 5 h in S1 ($P < 0.05$), neutrophil:lymphocyte ratio was lower in S2 at 0 h and S1 at 5 h, serum IL-1β concentration was greater in SC at 0 h and S1 at 5 h, and serum IL-6 in S2 at 0 h and S1 at 5 h (time x sow treatment, $P < 0.05$). Furthermore, serum urea nitrogen was greater in NC at 0 and 5 h than NPg ($P < 0.05$). In conclusion, Pg in sow and nursery diets improved weaned pig performance, impacted immune parameters and altered the inflammatory response to lipopolysaccharide stimulation.

Introduction

The abrupt change from sow's milk to solid feed, coupled with a change in environment, places a large amount of stress on young pigs. The weaned pig must quickly adjust from a liquid to solid diet, which may cause disruption of normal digestive tract function (Dong and Pluske, 2007). The removal of sow's milk at weaning creates an absence of compounds needed by the

intestinal epithelium, which may affect intestinal cell growth processes, cell differentiation, cell function, and immune function (Pluske, 2001). During the first week following weaning there can be reduced feed intake, which is detrimental to the intestinal tract of the pig (Dong and Pluske, 2007). A healthy digestive tract, coupled with a robust immune system should benefit performance of weaned pigs.

Yeast products have been demonstrated to enhance swine performance as both a probiotic or prebiotic with varied outcomes. Mannan oligosaccharides (MOS) and β-glucans are components of the yeast cell wall that are believed to confer beneficial effects on immune parameters in vertebrates (Soltanian et al., 2009). These components have been isolated and tested separately on swine. Improvement in growth performance (Davis et al., 2004b) and immune altering effects (White et al., 2002; Davis et al., 2004b) have been demonstrated for MOS. Immunomodulatory effects of β-glucans have also been demonstrated (Xiao et al., 2004; Soltanian et al., 2009). These benefits may also be conveyed through direct binding of pathogenic bacteria in the intestinal lumen, thereby flushing them out (Spring et al., 2000).

CitriStim (ADM Alliance Nutrition) is a whole yeast (*Pichia guilliermondii*; Pg) coproduct of citric acid extraction, containing the whole yeast cell and its components, which has been shown to have localized immunomodulatory effects in broilers (Shanmugasundaram and Selvaraj, 2012) but has not been extensively studied in the pig. Therefore, the objectives of this study were to determine the effect of supplementation of nursery diets with Pg on performance and immune parameters of weaned pigs from sows fed gestation and lactation diets with and without supplemental Pg. Additionally, the effects of an LPS challenge on the performance and immune parameters of these weaned pigs were also examined.

Materials and Methods

Animal management and experimental procedures conducted during this study were approved by the University of Arkansas Institutional Animal Care and Use Committee.

Animals. A total of 98 GPK 35 gilts and sows were allotted to 1 of 3 dietary treatments based on parity, and body weight at breeding. The 3 dietary treatments consisted of a gestation control diet (corn, soybean meal, and dried distillers grains with solubles-based; [SC]), or SC supplemented with 0.1 % Pg (S1) or 0.2% Pg (S2), with treatments maintained through gestation and lactation. Diets were formulated to meet or exceed NRC requirements for gestating and lactating gilts and sows (NRC, 1998). Methods and diets for the sow study can be found in Chapter III.

Nursery. Pigs from the first 2 breeding groups of gilts and sows were used to determine whether Pg fed to sows during gestation and lactation impacted growth performance of their progeny. The study was designed in a 3 (SC, S1, or S2) x 2 (nursery pigs with [NPg] or without [NC] Pg) factorial arrangement in an RCBD. For Exp. 1, a total of 336 weaned pigs (5.8 kg average BW) were individually weighed and grouped into weight blocks with stratification based on sex and litter and assigned to pens (7 pigs/pen, 8 replicates/treatment). Pigs were phase fed either a control diet (NC) or the control with Pg (NPg) inclusion at 0.2, 0.1, and 0.1% in phase 1 (7 d), phase 2 (14 d), and phase 3 (14 d), respectively. In Exp. 2, a total of 288 weaned pigs (6.4 kg average BW) were individually weighed and grouped into weight blocks with stratification based on sex and litter and assigned to pens (6 pigs/pen, 8 replicates/treatment). Pigs were phase fed, as in Exp. 1, either the NC or NPg (0.2% Pg in all phases) diet. Diets were formulated to meet or exceed NRC requirements (Table 1). Weaning occurred at approximately 21 d of age

and feed and water were provided *ad libitum* for both studies. Pigs were housed in a conventional nursery facility in elevated, wire floored pens.

Pigs were individually weighed at weaning and the end of each phase. Pen feed intake was also determined at the end of each phase. These measurements were used to calculate average daily gain, average daily feed intake, and gain to feed ratio.

Blood samples were collected on d 5 and 28 post-weaning for analysis of serum IgA, IgG, IgM, and haptoglobin, as well as whole blood white blood cell counts. Blood for serum analysis was collected into tubes containing clot activator (BD Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ). Blood samples were centrifuged at 1300 x g at room temperature for 20 min; serum was transferred into 5 mL polypropylene sample tubes and stored at -20˚C until analyzed for IgA, IgG, and IgM. Blood for determination of peripheral blood white blood cell counts was collected in K_2-EDTA tubes (BD Vacutainer) and analyzed using a blood hematology system (Hemavet 950 FS, Drew Scientific, Waterbury, CT).

Exp. 2A. To determine the impact of Pg fed to sows and their progeny on the immune response to an LPS challenge, 36 pigs from the second farrowing group were assigned to treatment in a 3 (SC, S1, or S2) x 2 (NC or NPg [0.2% Pg]) factorial arrangement in an RCBD. Pigs were individually penned in an isolated nursery facility at approximately 35 d of age and allowed a 3 d acclimation period. Pigs (40 d of age) were challenged with LPS (25 µg/kg BW intramuscular injection; 0 h) to induce an inflammatory immune response. Individual BW, ADFI, and rectal temperature were collected at -48, -24, 0 h, 24, and 48 h. Acute feed intake response (AFIR) was defined as the ADFI from 0 to 48 h after injection with LPS, divided by the baseline intake (-48 to 0 h) and multiplied by 100 to express the value as a percent. Blood samples were collected into vacuum tubes (Becton Dickinson and Company) at 0, 5, and 24 h for

analysis of peripheral blood white blood cell counts and serum concentrations of the inflammatory cytokines IL-1β and IL-6, urea nitrogen, serum amyloid A (SAA), C-reactive protein (CRP), and haptoglobin (HAP).

Chemical analysis. Colostrum, milk and serum IgA, IgG, and IgM (Pig ELISA Quantitation Set, Bethyl Laboratories, Inc., Montgomery, TX), serum SAA, CRP, and HAP (PHASE Range ELISA Kit, Tridelta Development Ltd., Kildare, Ireland) and serum IL-1β and IL-6 (Porcine DuoSet, R & D Systems, Inc., Minneapolis, MN) were analyzed with commercially available ELISA kits per manufacturer's instructions. Serum urea nitrogen was determined using a commercially available colorimetric kit [Urea Nitrogen (BUN) Reagent; Teco Diagnostics, Anaheim, CA] per the manufacturers' instructions.

Statistical analysis. Data were analyzed as a randomized complete block design with a 3 x 2 factorial arrangement of treatments. Pen served as the experimental unit, and data were analyzed using the GLM Procedure of SAS. Least squares means for main effects, and sow treatment x nursery treatment interactions are reported. Means were separated using LSD when main treatment effects were observed ($P < 0.05$) and PDIFF was used to separate LSMEANS for the significant ($P < 0.05$) sow treatment by nursery treatment interactions.

Results

Data for the 2 nursery experiments were analyzed separately due to the different levels of Pg inclusion in each of the 3 phases. Additionally, both main effect means of sow diet and nursery diet, as well as sow diet × nursery diet interactions, are presented as several interactions were observed in Exp. 1.

Performance

Exp. 1. As the level of Pg increased in gestation and lactation diets, there was a linear increase in ADG of weaned pigs during phase 1 ($P = 0.03$), phase 2 ($P = 0.04$), and the combined phase 1 and 2 period ($P < 0.01$; Table 2). During phase 1 a sow diet × nursery diet interaction was observed in ADG, with an increase in ADG in weaned pigs provided Pg as level of Pg inclusion increased in sow diets, but gain was similar in NC pig regardless of sow treatment (sow treatment × nursery treatment, $P = 0.01$; Table 2; Figure 1).

During phase 1, ADFI increased in weaned pigs provided Pg in the nursery compared to the control diet ($P = 0.005$). Additionally, a sow diet × nursery diet interaction was observed for ADFI during phase 1 ($P = 0.05$; Table 3; Figure 2) with a similar pattern as the interaction observed in phase 1 ADG. In phase 2, as the level of Pg increased in sow diets ADFI decreased in pigs fed NC, while intake for NPg for all sow treatments was not different (sow treatment × nursery treatment, $P = 0.06$; Figure 3). The gestation/lactation diet × nursery diet interaction continued during the combined phase 1 and 2 periods ($P = 0.04$; Figure 4), phase 3 ($P = 0.03$; Figure 5), and the overall nursery period ($P = 0.01$; Figure 6) with an increase in ADFI for pigs provided Pg in the nursery as the level of Pg increased in sow diets, and a decrease in ADFI as level of Pg increased in sow diets for NC pigs.

A linear increase in G:F with increasing level of Pg in sow diets was observed in phase 1 ($P = 0.04$), phase 2 ($P = 0.01$), the combined phase 1 and 2 periods ($P = 0.001$), and the overall nursery period ($P = 0.03$; Table 1). A sow diet × nursery diet interaction was observed for phase 2, phase 1 and 2 combined, and the overall nursery period ($P < 0.02$). Improvement in G:F was observed in weaned pigs provided the control diet as the level of Pg inclusion increased in sow diets, however, no differences were observed in G:F in weaned pigs provided Pg regardless of

sow diet (sow diet x nursery diet interaction; linear effect; Table 3; Figures 7, 8, 9, for phase 2, phase 1 and 2 combined, and overall; $P < 0.05$).

A linear increase in BW was observed at the end of phase 1 and phase 2 as the level of Pg increased in sow diets ($P = 0.01$; Table 1). Additionally, a sow diet × nursery diet interaction was observed for body weight with NPg pigs having increased BW at the end of phase 1 as the level of Pg inclusion increased in gilt and sow gestation and lactation diets (sow diet x nursery diet interaction; $P = 0.02$; Table 3; Figure 10).

Exp. 2. No interactions were observed in Exp. 2, so only main effects of gestation/lactation sow diet are reported (Table 4). There was a linear increase in ADG, ADFI, and G:F as the level of Pg increased in sow diets in phase 1, phase 2, and the overall nursery period ($P < 0.02$; Table 4). The interaction means are also presented for informational purposes (Table 5).

Interestingly, at the initiation of the study there was a linear decrease in BW of piglets as the level of Pg inclusion in sow diets increased ($P = 0.002$; Figure 11). However, by the end of phase 2, a linear increase in BW in piglets, as the level of Pg inclusion in sow diets increased, was observed, and was maintained through the end of the study ($P < 0.004$; Figure 11).

Immune system parameters

Exp. 1. Pigs from sows provided diets supplemented with 0.1% Pg tended to have an increased overall serum concentration of IgA (0.29, 0.41, and 0.31 mg/mL for SC, S1, and S2, respectively; sow quadratic, $P = 0.10$; Table 6), had an increased overall serum concentration of IgG (4.96, 5.96, and 5.38 mg/mL for SC, S1, and S2, respectively; sow quadratic, $P = 0.03$; Table 6), and tended to have decreased serum concentration of haptoglobin (1.85, 1.49, and 1.94

mg/mL in SC, S1, and S2, respectively; sow quadratic, $P = 0.08$; Table 6), than those from sows fed control or 0.2% Pg diets. Male weaned pigs had higher overall serum concentration of IgA than females ($P = 0.006$). On d 28, serum concentrations of IgA and IgM were higher, and concentrations of IgG and haptoglobin were lower than on d 5 ($P < 0.01$). Additionally, a day × sow treatment quadratic effect was observed for serum IgA with no difference among treatments on d 5, but increased serum IgA in pigs from S1 on d 28 compared to SC and S2 (day × sow quadratic; $P = 0.07$; Table 7).

On d 28, leukocyte and eosinophil concentrations were decreased in pigs from S1 compared to SC and S2 (sow quadratic, $P = 0.03$), lymphocyte concentration tended to decrease in pigs from S1 sows (sow quadratic, $P = 0.06$), and the percentage of eosinophils in the leukocyte population increased as level of Pg increased in sow diets (sow linear, $P = 0.06$; Table 8). Pigs fed NPg from S2 sows had a larger percentage of eosinophils in the leukocyte population on d 5 than NC (sow linear x nursery, $P = 0.01$; Table 9).

Exp. 2. On d 5 of the nursery study there was a tendency for a linear decrease in lymphocyte concentration and percentage of lymphocytes in the leukocyte population, and an increase in the neutrophil:lymphocyte ratio and percentage of neutrophils in the leukocyte population in piglets as the level of Pg inclusion in sow diets increased ($P \leq 0.1$; Table 10). Additionally, the monocyte concentration was greatest (sow quadratic, $P = 0.04$), the percentage of basophils in the leukocyte population was lowest (sow quadratic, $P = 0.03$), and the basophil concentration tended to be the least in pigs from S1 compared to pigs from sows fed SC or S2 (sow quadratic, $P = 0.1$). There was also a tendency for the percentage of monocytes in the leukocyte population to be lower in NPg compared to NC ($P = 0.06$).

On d 28, the percentage of both monocytes and basophils in the leukocyte population were decreased in pigs from S1 (sow quadratic, $P < 0.05$; Table 10). There was a tendency for pigs from S1 to have an increased neutrophil concentration and neutrophil:lymphocyte ratio, and decreased concentration of basophils (sow quadratic, $P \leq 0.09$). Additionally, the basophil concentration and percentage of basophils in the leukocyte population was reduced in NPg compared to NC ($P = 0.01$). There were no sow diet × nursery diet interactions in Exp. 2 (Table 11).

LPS challenge (Exp. 2A). Pigs from S1 tended to have increased ADG from -48 to -24 h (quadratic, $P = 0.07$) compared to SC and S2, and a tendency for a linear increase was observed from -48 to 0 h ($P = 0.09$; Table 12). A linear increase ($P \leq 0.04$) in ADFI was observed at -24 to 0 h, -48 to 0 h, and 24 to 48 h as the level of Pg increased in sow diets. Weaned pigs provided Pg had improved G:F at -48 to -24 h ($P = 0.02$). However, following challenge with LPS the acute feed intake response (ratio of intake from 0 to 48 h/intake from -48 to 0 h) was lower in weaned pigs provided Pg in the nursery diet ($P = 0.009$). Interestingly, there was a linear increase ($P < 0.01$) in rectal temperature with increasing Pg inclusion in sow diets at -48, -24, 0 and 5 h, and a quadratic response ($P < 0.01$) at 48 h with pigs from S1 being highest. However, at 5 h post-challenge there was a similar increase in rectal temperature ratio (difference in 5, 24, and 48 h rectal temperature, respectively, and baseline [-48, -24 , and 0 h]) among treatments, but at 24 h there was a linear decrease in rectal temperature ratio ($P < 0.01$) and at 48 h pigs from S2 tended to have the lowest ratio ($P = 0.06$).

Pigs from S1 sows had the greatest overall serum concentrations of IL-1β and IL-6 (sow quadratic, $P < 0.02$; Table 13). Additionally, overall serum concentrations of haptoglobin

linearly decreased as the level of Pg increased in sow diets (sow linear, $P = 0.05$). Weaned pigs fed a nursery diet supplemented with Pg had a lower overall serum concentration of BUN compared to those that received the control diet ($P = 0.003$).

There were time × sow diet interactions for serum concentrations of IL-1β, IL-6, and BUN (Table 14). The concentration of IL-1β was greater in SC piglets at 0 h, but by 5 h it was greatest in S1, but was similar among treatments by 24 h (time × sow diet, $P < 0.02$). Serum IL-6 was greater in S2 piglets at 0 h, but similar to IL-1β it was greatest in pigs from S1 at 5 h, and again was similar among treatments at 24 h (time × sow diet, $P < 0.01$). Furthermore, there was a tendency for an interaction for the serum concentration of BUN, with a greater concentration in pigs from SC at 0 h, but similar concentrations at 5 and 24 h post-challenge among treatments (time × sow diet, $P = 0.07$). Additionally, there was a time × nursery diet interaction for serum concentration of BUN (Table 15), with the concentration being greater at 0 and 5 h in weaned pigs provided the control diet compared to those provided diets supplemented with Pg, but similar at 24 h (time × nursery diet, $P = 0.05$).

Challenge with LPS changed the leukocyte profile of peripheral blood over the time course of the study. The concentration of leukocytes and lymphocytes decreased, and the neutrophil:lymphocyte ratio increased at 5 h post-LPS challenge, before returning to baseline at 24 h ($P < 0.01$; Table 16). The concentration of monocytes decreased, and the percentage of neutrophils in the leukocyte population increased at 5 h post-LPS challenge, but did not return to baseline at 24 h ($P < 0.01$). The percentage of lymphocytes in the leukocyte population decreased at 5 h post-LPS challenge before increasing to a level greater than baseline at 24 h. The percentage of eosinophils in the leukocyte population increased at 5 h post challenge before decreasing to a level lower than baseline at 24 h. The concentration of eosinophils and

basophils, as well as the percentage of basophils in the leukocyte population, were similar at 0 and 5 h, but were lower at 24 h ($P < 0.01$).

The leukocyte concentration was lowest (sow quadratic, $P = 0.02$; Table 16) and lymphocyte concentration tended to be lowest (sow quadratic, $P = 0.07$) in pigs from S1 compared with SC and S2. There was a linear increase in the monocyte concentration (sow linear, $P = 0.01$), a linear decrease in the neutrophil:lymphocyte ratio ($P = 0.03$), an increase in the percentage of monocytes in the leukocyte population ($P = 0.01$), and a tendency for a decrease in the percentage of neutrophils in the leukocyte population ($P = 0.09$) of weaned pig peripheral blood as the level of Pg inclusion increased in sow diets (Table 16).

There was a tendency for a time × sow diet interaction for the leukocyte concentration with the greatest concentration of leukocytes in pigs from S2, pigs from SC intermediate, and pigs from S1 having the lowest counts at 0 h, but those from SC being greatest at 5 h, and all treatments similar at 24 h (time × sow diet, $P = 0.08$; Table 17). Additionally, the neutrophil concentration was similar among treatments at 0 h, but were reduced at 5 h in S1 piglets compared to those from SC and S2, and was similar again at 24 h (time × sow diet, $P = 0.05$). The eosinophil concentration in peripheral blood was greatest in pigs from SC, intermediate in pigs from S2, and lowest in pigs from S1, however, at 5 h eosinophil concentration in pigs from SC was greatest, then similar among all treatment groups at 24 h (time × sow diet, $P = 0.04$). The neutrophil:lymphocyte ratio at 0 h was lowest in S2. At 5 h, the neutrophil:lymphocyte ratio was greatest in SC, then was similar among all treatments at 24 h (time x sow diet, $P < 0.01$). The percentage of neutrophils in the leukocyte population was similar among treatments at 0 and 24 h, but was greater in pigs from SC at 5 h compared to S1 and S2 (time × sow diet, $P = 0.01$). No interactions were observed between time and nursery diet ($P \geq 0.26$; Table 18).

Discussion

Yeast culture products derived from *Saccharomyces cerevisiae* contain mannan-oligosaccharides and β-glucans, components of the yeast cell wall, which may have beneficial effects on the performance and immune function of swine. The performance response to these substances in studies using weaned pigs has been varied, even when similar studies took place on the same farm (LeMieux et al., 2003; Davis et al., 2004a, Li et al., 2006). In this study we demonstrated the beneficial effects of feeding a whole yeast byproduct of citric acid production, *Pichia guilliermondii*, which contains MOS and β-glucans.

The benefits of Pg on performance of the weaned pig were most apparent when it was provided in gestation and lactation diets of the dam. In both Exp. 1 and 2, we observed a linear improvement in ADG in phases 1 and 2 as the level of Pg inclusion increased in gestation and lactation diets, and this carried to the overall nursery period in Exp. 2. Additionally, a linear improvement in G:F occurred in phase 1, phase 2, and the overall nursery period as Pg level increased in gestation and lactation diets. These improvements were evidenced in linear improvements in BW at the end of both studies as the level of Pg inclusion increased in gestation and lactation diets of the dam. When sows were provided an active dry yeast supplement from d 93 of gestation through lactation, pigs weaned from the supplemented sows had increased ADG and G:F at the end of a 28 d nursery study when compared to those from sows fed a control diet (Jurgens et al., 1997). Similar to our findings with Pg, an effect of supplementing diets with active dry yeast in the nursery was not reported, with the improvement observed in the nursery period attributed to sow diet.

Many of the benefits from MOS, β-glucan, or yeast culture products appear early in the nursery period and are often evident in early weaned, slower growing, or immune challenged

pigs. An improvement in ADG and G:F from d 0 to 14 and 0 to 21 was observed when weaned pigs were provided diets supplemented with phosphorylated mannans (Davis et al., 2004b). In the absence of pharmacological levels of copper, MOS improved ADG and G:F during phase 1, and MOS supplementation alone improved ADG and G:F in phase 3 and the overall 38 d nursery period when compared to weaned pigs provided a control diet (Davis et al., 2002). An increase in ADG and ADFI were observed in the first 14 d of a 28 d nursery study by Zhao et al. (2012) with the ADG improvement continuing through the entire study. Additional benefits to ADG (Rozeboom et al., 2005) and G:F (Rozeboom et al., 2005; Castillo et al., 2008) from providing MOS have been reported. However, others have reported no effects on weaned pig performance of MOS supplementation alone (LeMieux et al., 2003; Nochta et al., 2009; Nochta et al., 2010; Che et al., 2012) or in the presence of pharmacological levels of zinc (LeMieux et al., 2003). The improvement in performance from MOS may be due to improved digestibility or increased absorptive ability of the digestive tract (Nochta et al., 2010).

A mixed response to supplementation of nursery diets with β-glucans in performance has also been reported. An improvement in ADFI has also been reported by Hiss and Sauerwein (2003). Li et al. (2006) observed an improvement in ADG, with no effect on ADFI or G:F in one study, but an improvement in ADG and ADFI, but no effect on G:F in a second study when β-glucans extracted from *Saccharomyces cerevisiae* were provided in nursery diets of pigs weaned at 28 d of age. Finally, no response was observed in performance to a yeast cell wall extract containing β-glucan when provided in milk replacer from 7 d of age until weaning, and in nursery diets for a 4-wk nursery study (Sauerwein et al., 2007).

Performance response of weaned pigs to yeast culture has also been variable. Improvement in ADG and G:F, with no effect on ADFI, has been observed in pigs fed nursery

diets supplemented with yeast culture compared to control diets (van der Peet-Schwering et al., 2007). When soy or peanut hulls were included in starter diets, a decrease in G:F was observed, however, supplementation with yeast culture ameliorated this response and returned G:F to the level of control diets (Kornegay et al., 1995). Additionally, in the initial 3 wk grower phase following the nursery period, pigs provided the yeast culture had improved ADG, maintaining this improvement in pigs provided soyhulls. However, no improvement was evident when yeast culture was provided in nursery diets containing whey compared to those lacking whey (Kornegay et al., 1995). Diets supplemented with yeast culture had increased apparent DM, GE, and CP digestibility compared to control diets devoid of yeast culture supplementation (Shen et al., 2009). Shen and coworkers also reported an improvement in ADG and ADFI in pigs provided the yeast culture supplemented diet compared to those fed the control diet, but no differences in G:F. Prior to a challenge with *Salmonella*, there were no differences in performance parameters between pigs provided control diets, or diets supplemented with yeast culture (Price et al., 2010). However, following the challenge, pigs provided the supplemented diets had improved gain, and lower rectal temperatures, than those provided the control diets. Finally, weaned pigs challenged with *Escherichia coli* $K88^+$ had improved ADFI if provided diets supplemented with yeast culture compared to those provided the control diet (Kiarie et al., 2011).

In Exp. 1, pigs from sows receiving diets supplemented with 0.1% Pg had increased serum IgG and tended to have increased serum IgA and haptoglobin compared with the other treatment groups. Additionally, on d 5 there was no difference among treatment groups for serum concentration of IgA, but by d 28 IgA concentration was greatest in pigs from S1 sows compared to SC and S2. Sauerwein et al. (2007) postulated, based on other reports, serum IgA

may reflect IgA levels in the intestine. They found that the lower dosage (0.03 vs 0.3%) of yeast cell wall extract used in their study increased serum IgA. If true, and serum IgA can be used to gauge intestinal levels, pigs from S1 in the current study would have been provided improved intestinal defense against pathogens, which may partially explain their improved growth performance. When weaned pigs were provided diets supplemented with MOS, no differences were observed in levels of IgG (Castillo et al., 2008; Zhao et al., 2012), or IgA and IgM (Castillo et al., 2008). Increased concentration of haptoglobin may be found in pigs with inferior growth rates, and is usually negatively correlated with growth (Hiss and Sauerwein, 2003) though there was no difference in haptoglobin between weaned pigs provided diets supplemented with MOS or control diet in their study. Additionally, no difference in haptoglobin concentration was reported between pigs supplemented with β-glucan, or provided a control diet (Sauerwein et al., 2007).

When weaned pigs were provided diets supplemented with MOS the percentage of lymphocytes in the leukocyte population increased, and the percentage of neutrophils in the leukocyte population decreased (Davis et al., 2004b). In contrast, no differences were observed in lymphocyte or WBC concentration on d 0, 14, or 28 when weaned pigs were provided either MOS-supplemented or control diets (Zhao et al., 2012). In the current study differences among the WBC population were varied. In Exp. 1, pigs from S1 sows had a lower concentration of leukocytes, lymphocytes, monocytes, and eosinophils in the peripheral blood than pigs from SC or S2 sows. In Exp. 2, a linear decrease in lymphocyte concentration and increase in the neutrophil:lymphocyte ratio and percentage of neutrophils in the leukocyte population in weaned pigs as the level of Pg inclusion increased in sow diets were observed on d 5, and on d 28 the neutrophil concentration and neutrophil:lymphocyte ratio were greatest in weaned pigs from S1

sows. The increase in neutrophil concentration and the neutrophil:lymphocyte ration is associated with stress in the weaned pig (Stull et al., 1999). The increased concentration neutrophils observed in Exp. 2 may indicate an inflammatory response.

The physiological response in the pig following an LPS challenge has been well characterized in regards to the alterations in serum concentrations of various hormones and cytokines, changes in body temperature, and reductions in feed intake (Warren et al., 1997; Webel et al., 1997; Wright et al., 2000). Reductions in intake are dose-dependent in intensity and duration (Johnson and von Borell, 1994, Frank et al., 2005). When challenged with LPS, pigs from sows provided diets supplemented with Pg were better able to handle the immune challenge. Pigs from S1 gained more in the 48 h prior to challenge, and a linear increase in ADFI was observed prior to, and 24 hours post, LPS challenge as the level of Pg inclusion increased in sow gestation and lactation diets. There was a linear increase in rectal temperature prior to, and 5 h post LPS challenge. Interestingly, the difference in rectal temperature increase from baseline at 5 h was similar in all treatments, however, the decrease from baseline at 24 h following LPS challenge was greatest in pigs from S1 and S2, suggesting recovery to the febrile response occurred more rapidly in pigs from sows that were provided diets supplemented with Pg. Changes in the WBC population also improved in pigs from sows that were provided Pg as a decrease in the percentage of neutrophils in the leukocyte population and neutrophil:lymphocyte ratio was observed at 5 h post challenge in pigs from sows that were provided diets supplemented with Pg. However, the response of the inflammatory cytokines IL-1β and IL-6 were greatest in the serum of pigs from S1 sows at the same time point. Collier et al. (2011) supplemented nursery diets with active dry yeast and challenged with LPS. They found that the supplemented group had increased ADG compared to controls. The supplemented group also

had increased and increased WBC concentration prior to challenge, but was similar to pigs fed the control diet after challenge, and returned to baseline by 24 h post-challenge. The lymphocyte concentration increased in the control group, but no other differences in among the population of WBC was observed. Concentrations of serum IL-1β and IL-6 were similar between control and active dry yeast supplemented groups prior to LPS challenge, but increased for both cytokines to a greater extent in pigs provided the control diet by 2 h post challenge (Collier et al., 2011). A decrease in serum IL-6 (Li et al., 2005; Li et al., 2006) and increase in IL-10 (Li et al., 2005) was also observed in response to LPS when pigs were provided a diet supplemented with β-glucan compared to controls. Additionally, peripheral blood mononuclear cells from weaned pigs that were fed diets supplemented with β-glucans had lower concentrations of IL-6 in the supernatant at 3, 9, 12, and 18 h post-LPS challenge (Li et al., 2005). Finally, alveolar macrophages isolated from weaned pigs fed diets supplemented with MOS produced less TNFα and more IL-10 in response to LPS stimulation than those from pigs provided a control diet (Che et al., 2012). This response was also observed when naïve alveolar macrophages were cultured with MOS, compared to medium alone, and stimulated with LPS.

In conclusion, supplementation of standard gestation and lactation diets with Pg may be beneficial not only to sow reproductive performance (Chapter III), but continued inclusion of the whole yeast product, Pg, in nursery diets improved weaned pig performance, and this improvement may be additive. Inclusion of Pg in nursery diets resulted in an improvement in average daily gain as well as feed intake in pigs fed Pg as the level of Pg increased in sow diets. Finally, Pg inclusion in sow diets was also beneficial to efficiency in pigs receiving control nursery diets. It appears inclusion of Pg during gestation and lactation, especially at 0.1%, impacted immune response to LPS as these pigs had a decrease in the concentration of

neutrophils in the blood, as well as the neutrophil:lymphocyte ratio, and a swift, but robust, increase in the inflammatory cytokines IL-1β and IL-6 at 5 h post-challenge.

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Table 1. Composition (as-fed) of nursery diets[1]

Ingredients (%)	Phase 1	Phase 2	Phase 3
Corn	40.66	50.34	56.70
Soybean meal, 48%	19.50	31.50	24.50
Dried distillers grains with solubles	—	5.00	15.00
Oat groats	10.00	—	—
Poultry fat	1.50	1.00	—
L-Lysine	0.30	0.27	0.30
DL-Methionine	0.17	0.11	0.03
L-Threonine	0.11	0.08	0.07
L-Tryptophan	0.01	—	—
Whey	16.45	8.00	—
Plasma protein	4.00	—	—
Fish meal	4.00	—	—
Monocalcium phosphate	0.89	1.17	1.19
Calcium carbonate	0.75	1.08	1.28
Salt	0.57	0.45	0.40
ADM Premidex[2]	0.20	0.20	0.10
Zinc oxide	0.35	0.28	—
Copper sulfate	0.10	0.10	—
Vitamin premix[3]	0.25	0.25	`
Mineral premix[4]	0.15	0.15	0.15
Ethoxyquin	0.03	0.03	0.03
Calculated composition[5]			
ME (kcal/kg)	1.52	1.50	1.50
CP (%)	22.18	21.80	20.64
SID Lysine (%)	1.43	1.26	1.10
Available P (%)	0.52	0.40	0.40
Ca (%)	0.89	0.84	0.85
SID M+C:Lys	58	58	58
SID Thr:Lys	62	62	63
SID Trp:Lys	17	18	17
SID Ile:Lys	56	64	65
SID Val:Lys	66	70	74

[1]Control diets for nursery. Exp. 1 - Pg added at 0.2, 0.1, and 0.1% in Phase 1, 2, and 3, respectively at the expense of corn.; Exp. 2 - Pg added at 0.2% in all phases at the expense of corn.

[2] ADM Alliance Nutrition, Quincy, IL.

[3]The vitamin premix provided the following per kg of complete diet: 397.5 mg of Ca, 11,022.9 IU of vitamin A, 1,377.9 IU of vitamin D_3, 44.09 IU of vitamin E, 0.0386 mg vitamin B_{12}, 4.41 mg of menadione, 8.27 mg of riboflavin, 27.56 mg of D-pantothenic acid, and 49.6 mg of niacin.

[4]The mineral premix provided the following per kg of complete diet: 84 mg of Ca, 165 mg of Fe, 165 mg of Zn, 39.6 mg of Mn, 16.5 mg of Cu, 0.3 mg of I, and 0.3 mg of Se.

[5]ME – metabolizable energy; CP – crude protein; SID – standard ileal digestible.

Table 2. Main effects of *Pichia guilliermondii* (Pg) on nursery growth performance (Exp. 1).

	Sow Treatment[1]				*P*-value		Contrasts	
Variable	SC	S1	S2	SEM	Sow diet	Sow × nursery	Linear	Quadratic
ADG, g								
Phase 1	124.4	139.1	147.3	7.4	0.10	0.01	0.03	0.72
Phase 2	342.8	375.2	375.6	11.1	0.07	0.39	0.04	0.24
Phase 1-2	269.9[b]	297.1[a]	299.5[a]	7.7	0.02	0.37	0.01	0.19
Phase 3	427.4	438.9	430.7	16.1	0.87	0.58	0.88	0.62
Overall	335.6	353.5	352.0	8.2	0.24	0.28	0.16	0.34
ADFI, g/d								
Phase 1	174.3	193.7	187.1	6.1	0.08	0.05	0.14	0.09
Phase 2	506.1	507.3	496.7	15.0	0.86	0.06	0.66	0.75
Phase 1-2	393.9	402.2	393.5	11.2	0.82	0.04	0.98	0.54
Phase 3	779.8	764.4	770.0	20.2	0.86	0.03	0.73	0.67
Overall	543.3	546.0	544.1	12.2	0.99	0.01	0.96	0.88
Gain:feed								
Phase 1	0.680	0.719	0.784	0.035	0.11	0.34	0.04	0.75
Phase 2	0.670[b]	0.742[a]	0.762[a]	0.023	0.02	0.01	0.01	0.36
Phase 1-2	0.672[b]	0.738[a]	0.766[a]	0.019	0.003	0.03	0.001	0.42
Phase 3	0.551	0.574	0.558	0.019	0.70	0.35	0.79	0.42
Overall	0.605[b]	0.646[a]	0.649[a]	0.014	0.05	0.02	0.03	0.27
Weight, kg								
Initial	5.78	5.81	5.88	0.06	0.51	0.91	0.26	0.79
Phase 1	6.65[b]	6.78[b]	6.91[a]	0.06	0.03	0.02	0.01	0.98
Phase 2	11.45[b]	12.05[a]	12.17[a]	0.19	0.02	0.42	0.01	0.30
Phase 3	17.52	18.18	18.20	0.32	0.24	0.32	0.14	0.41

[1]SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg.

[a.b] Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 2 (cont). Main effects of *Pichia guilliermondii* (Pg) on nursery growth performance (Exp. 1).

	Nursery Treatment[1]			*P*-value	
Variable	**NC**	**NPg[2]**	**SEM**	**Nursery diet**	**Sow × nursery**
ADG, g					
Phase 1	130.2	143.7	6.0	0.12	0.01
Phase 2	369.0	360.1	9.1	0.50	0.39
Phase 1-2	289.5	288.2	6.3	0.88	0.37
Phase 3	431.5	433.1	13.1	0.93	0.58
Overall	346.3	347.7	6.7	0.88	0.28
ADFI, g/d					
Phase 1	174.5	195.6	4.9	0.005	0.05
Phase 2	503.3	503.4	12.3	0.99	0.06
Phase 1-2	392.9	400.2	9.2	0.58	0.04
Phase 3	779.8	762.9	16.5	0.47	0.03
Overall	545.1	543.8	10.0	0.93	0.01
Gain:feed					
Phase 1	0.729	0.727	0.028	0.95	0.34
Phase 2	0.734	0.715	0.019	0.46	0.01
Phase 1-2	0.733	0.718	0.016	0.49	0.03
Phase 3	0.556	0.566	0.016	0.66	0.35
Overall	0.633	0.634	0.011	0.97	0.02
Weight, kg					
Initial	5.82	5.82	0.05	1.00	0.91
Phase 1	6.73	6.83	0.05	0.20	0.02
Phase 2	11.90	11.87	0.15	0.88	0.42
Phase 3	17.94	17.99	0.26	0.89	0.32

[1]NC – Nursery control diet; NPg – Nursery control diet + Pg.
[2] Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3).

Table 3. Interactive effects of *Pichia guilliermondii* (Pg) on nursery growth performance (Exp. 1).

	SC[1]		S1		S2		SEM	Interaction	Sow × nursery	
	NC	NPg[2]	NC	NPg	NC	NPg		*P*-value	Linear	Quadratic
ADG, g										
Phase 1	134.9^{bc}	113.9^{c}	130.7^{bc}	147.5^{ab}	124.9^{bc}	169.8^{a}	10.4	0.01	0.003	0.79
Phase 2	338.2	347.4	391.7	358.7	377.0	374.3	15.7	0.39	0.71	0.19
Phase 1-2	269.8	270.1	305.8	288.3	292.9	306.1	10.8	0.37	0.56	0.20
Phase 3	426.9	427.8	449.9	427.9	417.8	443.6	22.7	0.58	0.59	0.37
Overall	333.2	337.9	362.9	344.2	342.9	361.1	11.6	0.28	0.56	0.14
ADFI, g/d										
Phase 1	173.7^{c}	174.9^{c}	184.9^{bc}	202.5^{ab}	165.0^{c}	209.3^{a}	8.6	0.05	0.01	0.73
Phase 2	534.6^{a}	477.6^{ab}	501.5^{ab}	513.0^{ab}	473.7^{b}	519.6^{ab}	21.3	0.06	0.02	0.65
Phase 1-2	413.0^{ab}	374.8^{ab}	394.9^{ab}	409.5^{ab}	370.8^{b}	416.2^{a}	15.9	0.04	0.01	0.69
Phase 3	828.6^{a}	730.9^{b}	770.1^{ab}	758.7^{ab}	740.8^{b}	799.1^{ab}	28.5	0.03	0.01	0.87
Overall	573.7^{a}	512.9^{b}	542.8^{ab}	549.2^{ab}	518.8^{b}	569.4^{a}	17.3	0.01	0.003	0.70
Gain:feed										
Phase 1	0.721	0.639	0.711	0.726	0.754	0.814	0.049	0.34	0.15	0.76
Phase 2	0.622^{c}	0.717^{ab}	0.782^{ab}	0.702^{bc}	0.799^{a}	0.725^{ab}	0.032	0.01	0.01	0.11
Phase 1-2	0.637^{c}	0.708^{bc}	0.770^{ab}	0.706^{bc}	0.793^{a}	0.740^{ab}	0.027	0.03	0.02	0.13
Phase 3	0.524	0.579	0.582	0.565	0.563	0.554	0.027	0.35	0.24	0.39
Overall	0.572^{b}	0.638^{a}	0.665^{a}	0.627^{ab}	0.663^{a}	0.636^{a}	0.020	0.02	0.03	0.10
Weight, kg										
Initial	5.79	5.77	5.82	5.80	5.85	5.90	0.08	0.91	0.72	0.79
Phase 1	6.73^{b}	6.57^{b}	6.74^{b}	6.83^{b}	6.73^{b}	7.09^{a}	0.09	0.02	0.01	0.96
Phase 2	11.46	11.44	12.25	11.85	12.01	12.33	0.27	0.41	0.53	0.24
Phase 3	17.45	17.59	18.52	17.84	17.86	18.54	0.45	0.32	0.55	0.17

[1]SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg; NC – Nursery control diet; NPg – Nursery control + Pg.

[2]CitriStim added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) at the expense of corn.

[a,b,c]Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 4. Main effects of *Pichia guilliermondii* (Pg) on nursery growth performance (Exp. 2).

	Sow Treatment[1]				*P*-value		Contrasts	
Variable	**SC**	**S1**	**S2**	**SEM**	**Sow Diet**	**Sow × Nursery**	**Linear**	**Quadratic**
ADG, g								
Phase 1	25.6[b]	33.5[b]	67.9[a]	9.9	0.01	0.77	0.004	0.28
Phase 2	224.1[c]	281.7[b]	334.4[a]	14.8	<0.0001	0.93	<0.0001	0.89
Phase 1-2	158.7[c]	198.7[b]	246.1[a]	12.0	<0.0001	0.92	<0.0001	0.80
Phase 3	415.7	406.3	429.4	15.9	0.59	0.65	0.55	0.41
Overall	262.8[b]	281.7[b]	323.0[a]	12.2	0.004	0.83	0.001	0.46
ADFI, g/d								
Phase 1	96.7[b]	110.6[ab]	122.2[a]	5.2	0.005	0.83	0.001	0.86
Phase 2	334.5[b]	399.2[a]	426.7[a]	15.6	0.0005	0.71	0.0002	0.34
Phase 1-2	249.8[b]	297.7[a]	318.1[a]	11.1	0.0003	0.82	<0.0001	0.32
Phase 3	701.7	681.2	730.6	21.3	0.27	0.68	0.34	0.19
Overall	414.2[b]	440.8[ab]	469.2[a]	13.3	0.02	0.76	0.006	0.96
Gain:feed								
Phase 1	0.197[b]	0.253[ab]	0.502[a]	0.089	0.04	0.43	0.02	0.38
Phase 2	0.631[b]	0.674[b]	0.757[a]	0.019	0.0001	0.47	<0.0001	0.38
Phase 1-2	0.575[b]	0.617[b]	0.716[a]	0.021	0.0001	0.46	<0.0001	0.28
Phase 3	0.582	0.590	0.567	0.013	0.46	0.94	0.42	0.35
Overall	0.582[b]	0.604[ab]	0.634[a]	0.011	0.007	0.74	0.002	0.78
Weight, kg								
Initial	6.56[a]	6.43[ab]	6.31[b]	0.05	0.002	0.92	0.0004	0.92
Phase 1	6.74	6.67	6.79	0.07	0.53	0.80	0.64	0.31
Phase 2	9.90[b]	10.59[b]	11.49[a]	0.26	0.0004	0.96	<0.0001	0.73
Phase 3	15.78[b]	16.28[b]	17.64[a]	0.43	0.01	0.83	0.004	0.41

[1]SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg.

[a,b,c]Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 4 (Cont). Main effects of *Pichia guilliermondii* (Pg) on nursery growth performance (Exp. 2).

	Nursery Treatment[1]			*P*-value	
Variable	**NC**	**NPg[2]**	**SEM**	**Nursery diet**	**Sow × nursery**
ADG, g					
Phase 1	51.9	32.7	8.1	0.10	0.77
Phase 2	283.6	276.6	12.1	0.68	0.93
Phase 1-2	205.6	196.8	9.8	0.53	0.92
Phase 3	415.5	418.8	13.0	0.86	0.65
Overall	292.8	285.6	10.0	0.61	0.83
ADFI, g/d					
Phase 1	111.5	108.1	4.2	0.57	0.83
Phase 2	393.9	379.7	12.7	0.43	0.71
Phase 1-2	293.1	283.9	9.0	0.47	0.82
Phase 3	695.5	713.5	17.4	0.47	0.68
Overall	440.7	442.1	10.9	0.93	0.76
Gain:feed					
Phase 1	0.420	0.215	0.073	0.05	0.43
Phase 2	0.692	0.683	0.015	0.68	0.47
Phase 1-2	0.649	0.623	0.017	0.31	0.46
Phase 3	0.579	0.580	0.011	0.98	0.94
Overall	0.613	0.601	0.009	0.33	0.74
Weight, kg					
Initial	6.44	6.43	0.04	0.86	0.92
Phase 1	6.80	6.66	0.06	0.10	0.80
Phase 2	10.76	10.56	0.21	0.50	0.96
Phase 3	16.70	16.43	0.35	0.59	0.83

[1]NC – Nursery control diet; NPg – Nursery control + Pg.
[2]Pg added at 0.2% at the expense of corn.
[a,b,c]Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 5. Interactive effects of *Pichia guilliermondii* (Pg) on nursery growth performance (Exp. 2).

	SC[1]		S1		S2		SEM	Interaction
	NC	NPg[2]	NC	NPg	NC	NPg		*P*-value
ADG, g								
Phase 1	40.7	10.5	38.8	28.2	76.4	59.4	14.0	0.77
Phase 2	229.6	218.5	287.9	275.6	333.2	335.6	21.0	0.93
Phase 1-2	165.3	152.2	204.9	192.5	246.5	245.7	17.0	0.92
Phase 3	420.9	410.5	392.5	420.0	433.1	425.8	22.6	0.65
Overall	271.7	253.8	280.0	283.5	326.7	319.3	17.4	0.83
ADFI, g/d								
Phase 1	95.8	97.5	113.4	107.7	125.3	119.1	7.3	0.83
Phase 2	346.4	322.6	395.8	402.6	439.4	413.9	22.0	0.71
Phase 1-2	257.4	242.1	296.5	298.8	325.5	310.7	15.7	0.82
Phase 3	703.1	700.2	657.1	705.3	726.3	734.8	30.1	0.67
Overall	417.9	410.5	432.1	449.6	472.3	466.1	18.9	0.76
Gain:feed								
Phase 1	0.394	0.000	0.299	0.206	0.567	0.438	0.126	0.43
Phase 2	0.636	0.627	0.695	0.652	0.745	0.769	0.027	0.47
Phase 1-2	0.596	0.555	0.643	0.591	0.707	0.725	0.030	0.46
Phase 3	0.585	0.579	0.587	0.594	0.567	0.567	0.019	0.94
Overall	0.594	0.571	0.611	0.597	0.634	0.634	0.015	0.74
Weight, kg								
Initial	6.55	6.56	6.43	6.43	6.33	6.29	0.06	0.92
Phase 1	6.84	6.64	6.70	6.64	6.87	6.71	0.10	0.80
Phase 3	16.08	15.48	16.21	16.34	17.80	17.48	0.61	0.83

[1]SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg; NC – Nursery control diet; NPg – Nursery control + Pg.

[2]Pg added at 0.2% at the expense of corn.

[a,b,c]Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 6. Main effects of *Pichia guilliermondii* (Pg) on serum IgA, IgG, IgM and haptoglobin concentrations of nursery pigs (Exp. 1).

	Sow Treatment[1]			Nursery Treatment[1]		Sex	
Item	**SC**	**S1**	**S2**	**NC**	**NPg[2]**	**Female**	**Male**
IgA[3]	0.29 ± 0.05	0.41 ± 0.05	0.31 ± 0.04	0.35 ± 0.04	0.33 ± 0.04	0.25 ± 0.04	0.43 ± 0.04
IgG[3]	4.96 ± 0.35[b]	5.96 ± 0.34[a]	5.38 ± 0.34[ab]	5.29 ± 0.29	5.58 ± 0.30	5.48 ± 0.29	5.39 ± 0.30
IgM[3]	1.38 ± 0.20	1.67 ± 0.20	1.69 ± 0.20	1.64 ± 0.17	1.52 ± 0.17	1.48 ± 0.17	1.68 ± 0.17
Haptoglobin[3]	1.85 ± 0.19	1.49 ± 0.19	1.94 ± 0.19	1.78 ± 0.15	1.74 ± 0.15	1.83 ± 0.15	1.68 ± 0.15

	Time		*P*-value[4]					Contrasts			
			Sex	**Time**	**ST**	**NT**	**ST x NT**	**Time x ST x NT**	**L ST**	**Q ST**	**Time x L ST**	**Time x Q ST**
IgA[3]	d 5	d 28	0.006	< 0.0001	0.24	0.83	0.46	0.50	0.77	0.10	0.84	0.07
IgG[3]	0.12 ± 0.04	0.56 ± 0.05	0.82	0.004	0.07	0.40	0.30	0.14	0.33	0.03	0.77	0.71
IgM[3]	5.96 ± 0.29	4.91 ± 0.30	0.30	0.007	0.34	0.57	0.49	0.40	0.19	0.53	0.23	0.52
Haptoglobin[3]	1.31 ± 0.17	1.85 ± 0.17	0.50	0.001	0.20	0.87	0.54	0.98	0.75	0.08	0.17	0.64

[1]SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg; NC – Nursery control diet; NPg – Nursery control + Pg.

[2]Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3).

[3] Serum concentration mg/mL.

[4] ST – sow diet; NT – nursery diet; L – linear contrast; Q – quadratic contrast.

[a.b] Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 7. Interactive effect of day by sow diet on serum IgA, IgG, IgM and haptoglobin concentrations of nursery pigs on d 5 and 28 of the nursery period (Exp. 1).

	d 5			d 28			*P*-value
	SC[1]	S1	S2	SC	S1	S2	Time x sow diet
IgA, mg/mL[2]	0.12 ± 0.07	0.11 ± 0.07	0.12 ± 0.07	0.47 ± 0.08	0.72 ± 0.08	0.50 ± 0.08	0.17
IgG, mg/mL	5.50 ± 0.45	6.58 ± 0.45	5.80 ± 0.45	4.42 ± 0.50	5.34 ± 0.45	4.96 ± 0.45	0.89
IgM, mg/mL	1.01 ± 0.26	1.31 ± 0.28	1.61 ± 0.25	1.75 ± 0.25	2.03 ± 0.26	1.78 ± 0.26	0.39
Haptoglobin, mg/mL	2.10 ± 0.26	1.81 ± 0.26	2.54 ± 0.26	1.61 ± 0.26	1.17 ± 0.26	1.33 ± 0.26	0.36

[1]SC - Sow control diet; S1 – Sow control diet + 0.1% *Pichia guilliermondii* (Pg); S2 - Sow control diet + 0.2% Pg.
[2]Time × Sow diet quadratic effect (*P* = 0.07).

Table 8. Main effects of *Pichia guilliermondii* (Pg) on concentration and proportions among peripheral blood leukocytes of nursery pigs on d 5 and 28 of the nursery period (Exp. 1).

	Sow Treatment[1]			*P*-value		Contrasts	
d 5	SC	S1	S2	Sow	S x N	Linear	Quadratic
WBC (K/uL)[2]	16.19 ± 1.54	16.60 ± 1.28	14.67 ± 1.23	0.53	0.62	0.45	0.48
NE (K/uL)	6.33 ± 0.72	6.41 ± 0.60	5.41 ± 0.58	0.43	0.80	0.33	0.48
LY (K/uL)	6.02 ± 0.71	6.28 ± 0.59	5.51 ± 0.57	0.64	0.54	0.58	0.49
MO (K/uL)	1.02 ± 0.08	0.91 ± 0.06	0.97 ± 0.06	0.55	0.88	0.59	0.31
EO (K/uL)	2.69 ± 0.48	2.86 ± 0.40	2.65 ± 0.39	0.93	0.07	0.96	0.71
BA (K/uL)	0.14 ± 0.04	0.14 ± 0.03	0.13 ± 0.03	0.98	0.46	0.89	0.91
NE:LY	1.06 ± 0.23	1.30 ± 0.19	1.07 ± 0.18	0.61	0.34	0.96	0.33
NE (%)	38.41 ± 3.02	39.94 ± 2.50	36.40 ± 2.41	0.60	0.82	0.61	0.43
LY (%)	38.13 ± 2.79	37.09 ± 2.31	38.14 ± 2.22	0.94	0.15	1.00	0.72
MO (%)	6.37 ± 0.62	5.87 ± 0.51	7.15 ± 0.49	0.21	0.34	0.33	0.18
EO (%)	16.24 ± 1.99	16.35 ± 1.65	17.49 ± 1.59	0.84	0.01	0.63	0.81
BA (%)	0.84 ± 0.21	0.76 ± 0.18	0.82 ± 0.17	0.94	0.19	0.93	0.73
d 28							
WBC (K/uL)	22.04 ± 1.54^{ab}	19.53 ± 1.54^{b}	25.76 ± 1.60^{a}	0.03	0.46	0.10	0.03
NE (K/uL)	7.65 ± 0.54	7.21 ± 0.54	8.50 ± 0.56	0.26	0.20	0.28	0.20
LY (K/uL)	9.01 ± 0.75	7.76 ± 0.75	10.06 ± 0.78	0.12	0.88	0.34	0.06
MO (K/uL)	1.64 ± 0.17	1.33 ± 0.17	1.83 ± 0.18	0.14	0.23	0.45	0.07
EO (K/uL)	3.55 ± 0.45^{b}	3.02 ± 0.45^{b}	5.08 ± 0.47^{a}	0.01	0.75	0.02	0.03
BA (K/uL)	0.19 ± 0.04	0.20 ± 0.04	0.28 ± 0.04	0.21	0.40	0.11	0.44
NE:LY	0.88 ± 0.07	0.98 ± 0.07	0.90 ± 0.07	0.56	0.51	0.82	0.30
NE (%)	35.44 ± 1.62	37.19 ± 1.62	33.11 ± 1.68	0.23	0.67	0.32	0.15
LY (%)	40.99 ± 1.49	39.36 ± 1.49	38.87 ± 1.54	0.58	0.55	0.33	0.76
MO (%)	7.26 ± 0.42	6.86 ± 0.42	7.18 ± 0.43	0.78	0.53	0.90	0.49
EO (%)	15.48 ± 1.52	15.55 ± 1.52	19.77 ± 1.58	0.10	0.92	0.06	0.28
BA (%)	0.83 ± 0.14	1.03 ± 0.14	1.07 ± 0.14	0.42	0.36	0.22	0.65

[1]SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg.

[2] WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/µL) – 1,000 cells/µL; (%) - percentage of total WBC.

Table 8 (cont.). Main effects of *Pichia guilliermondii* (Pg) on concentration and proportions among peripheral blood leukocytes of nursery pigs on d 5 and 28 of the nursery period (Exp. 1).

	Nursery Treatment		*P*-value		Sow Treatment	
d 5	NC	NPg[1]	Nursery	S x N	Linear	Quadratic
WBC (K/uL)[2]	14.90 ± 1.03	16.74 ± 1.18	0.25	0.62	0.45	0.48
NE (K/uL)	5.55 ± 0.48	6.55 ± 0.55	0.18	0.80	0.33	0.48
LY (K/uL)	5.57 ± 0.48	6.31 ± 0.55	0.31	0.54	0.58	0.49
MO (K/uL)	0.95 ± 0.05	0.99 ± 0.06	0.57	0.88	0.59	0.31
EO (K/uL)	2.72 ± 0.32	2.75 ± 0.37	0.95	0.07	0.96	0.71
BA (K/uL)	0.12 ± 0.03	0.15 ± 0.03	0.50	0.46	0.89	0.91
NE:LY	1.17 ± 0.15	1.11 ± 0.17	0.81	0.34	0.96	0.33
NE (%)	37.72 ± 2.02	38.78 ± 2.31	0.73	0.82	0.61	0.43
LY (%)	37.76 ± 1.86	37.81 ± 2.13	0.99	0.15	1.00	0.72
MO (%)	6.56 ± 0.41	6.36 ± 0.47	0.76	0.34	0.33	0.18
EO (%)	17.19 ± 1.33	16.20 ± 1.52	0.63	0.01	0.63	0.81
BA (%)	0.76 ± .014	0.85 ± 0.16	0.69	0.19	0.93	0.73
d 28						
WBC (K/uL)	22.52 ± 1.25	22.36 ± 1.29	0.93	0.46	0.10	0.03
NE (K/uL)	7.99 ± 0.44	7.58 ± 0.45	0.53	0.20	0.28	0.20
LY (K/uL)	8.66 ± 0.61	9.22 ± 0.63	0.53	0.88	0.34	0.06
MO (K/uL)	1.58 ± 0.14	1.62 ± 0.14	0.87	0.23	0.45	0.07
EO (K/uL)	4.05 ± 0.37	3.72 ± 0.38	0.52	0.75	0.02	0.03
BA (K/uL)	0.23 ± 0.03	0.23 ± 0.03	1.00	0.40	0.11	0.44
NE:LY	0.98 ± 0.06	0.86 ± 0.06	0.14	0.51	0.82	0.30
NE (%)	36.15 ± 1.32	34.35 ± 1.36	0.35	0.67	0.32	0.15
LY (%)	38.59 ± 1.21	40.89 ± 1.24	0.19	0.55	0.33	0.76
MO (%)	7.04 ± 0.34	7.16 ± 0.35	0.81	0.53	0.90	0.49
EO (%)	17.25 ± 1.25	16.62 ± 1.28	0.72	0.92	0.06	0.28
BA (%)	0.97 ± 0.11	0.98 ± 0.11	0.94	0.36	0.22	0.65

[1] NC – Nursery control diet; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3).

[2] WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/µL) – 1,000 cells/µL; (%) - percentage of total WBC.

Table 9. Interactive effects of sow and nursery *Pichia guilliermondii* (Pg) treatments on concentration and proportions among peripheral blood leukocytes of nursery pigs on d 5 and 28 of the nursery period (Exp. 1).

d 5	SC-NC[1]	SC-NPg[2]	S1-NC	S1-NPg	S2-NC	S2-NPg	S x N
WBC (K/uL)[2]	16.31 ± 1.74	16.08 ± 2.55	15.55 ± 1.87	17.64 ± 1.74	12.83 ± 1.74	16.51 ± 1.74	0.62
NE (K/uL)	6.04 ± 0.82	6.62 ± 1.20	6.02 ± 0.88	6.79 ± 0.82	4.58 ± 0.82	6.24 ± 0.82	0.80
LY (K/uL)	6.02 ± 0.80	6.02 ± 1.18	5.36 ± 0.86	7.20 ± 0.80	5.32 ± 0.80	5.70 ± 0.80	0.54
MO (K/uL)	0.98 ± 0.09	1.07 ± 0.13	0.89 ± 0.9	0.94 ± 0.09	0.97 ± 0.09	0.97 ± 0.09	0.88
EO (K/uL)[L]	3.18 ± 0.54	2.19 ± 0.80	3.14 ± 0.59	2.58 ± 0.54	1.83 ± 0.54	3.48 ± 0.54	0.07
BA (K/uL)	0.09 ± 0.04	0.19 ± 0.06	0.15 ± 0.05	0.13 ± 0.04	0.13 ± 0.04	0.13 ± 0.04	0.46
NE:LY	1.01 ± 0.26	1.10 ± 0.38	1.55 ± 0.28	1.05 ± 0.26	0.95 ± 0.26	1.19 ± 0.26	0.34
NE (%)	36.44 ± 3.41	40.39 ± 4.99	40.29 ± 3.67	39.59 ± 3.41	36.45 ± 3.41	36.35 ± 3.41	0.82
LY (%)[#]	38.54 ± 3.14	37.72 ± 4.61	33.63 ± 3.38	40.54 ± 3.14	41.12 ± 3.14	35.16 ± 3.14	0.15
MO (%)	6.08 ± 0.70	6.66 ± 1.02	5.72 ± 0.75	6.02 ± 0.70	7.89 ± 0.70	6.42 ± 0.70	0.34
EO (%)[L]	18.44 ± 2.25[ab]	14.04 ± 3.29[ab]	19.44 ± 2.12[ab]	13.25 ± 2.25[b]	13.67 ± 2.25[b]	21.31 ± 2.25[a]	0.01
BA (%)	0.50 ± 0.24	1.19 ± 0.35	0.91 ± 0.26	0.60 ± 0.24	0.87 ± 0.24	0.77 ± 0.24	0.19
d 28							
WBC (K/uL)	20.61 ± 2.17	23.47 ± 2.17	19.88 ± 2.17	17.18 ± 2.17	27.06 ± 2.17	24.45 ± 2.34	0.46
NE (K/uL)*	7.10 ± 0.77	8.20 ± 0.77	7.51 ± 0.77	6.91 ± 0.77	9.36 ± 0.77	7.64 ± 0.82	0.20
LY (K/uL)	8.44 ± 1.06	9.58 ± 1.06	7.71 ± 1.06	7.81 ± 1.06	9.84 ± 1.06	10.28 ± 1.14	0.88
MO (K/uL)*	1.40 ± 0.24	1.88 ± 0.24	1.33 ± 0.24	1.34 ± 0.24	2.02 ± 0.24	1.63 ± 0.26	0.23
EO (K/uL)	3.52 ± 0.63	3.58 ± 0.63	3.12 ± 0.63	2.93 ± 0.63	5.53 ± 0.63	4.64 ± 0.68	0.75
BA (K/uL)	0.15 ± 0.06	0.24 ± 0.06	0.22 ± 0.06	0.18 ± 0.06	0.31 ± 0.06	0.26 ± 0.06	0.40
NE:LY	0.87 ± 0.10	0.88 ± 0.10	1.08 ± 0.10	0.88 ± 0.10	0.99 ± 0.10	0.81 ± 0.11	0.51
NE (%)	35.16 ± 2.29	35.72 ± 2.29	38.86 ± 2.29	35.52 ± 2.29	34.41 ± 2.29	31.80 ± 2.46	0.67
LY (%)	41.10 ± 2.10	40.89 ± 2.10	38.02 ± 2.10	40.70 ± 2.10	36.66 ± 2.10	41.08 ± 2.26	0.55
MO (%)	6.99 ± 0.59	7.54 ± 0.59	6.63 ± 0.59	7.10 ± 0.59	7.52 ± 0.59	6.85 ± 0.63	0.53
EO (%)	16.09 ± 2.15	14.86 ± 2.15	15.37 ± 2.15	15.74 ± 2.15	20.29 ± 2.15	19.25 ± 2.32	0.92
BA (%)	0.66 ± 0.19	1.00 ± 0.19	1.12 ± 0.19	0.94 ± 0.19	1.13 ± 0.19	1.02 ± 0.21	0.36

[1] SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg; NC – Nursery control diet; NPg – Nursery control + Pg. Pg was added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3).

[2] WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/ µL) – 1,000 cells/µL; (%) - percentage of total WBC.

[L] Sow diet × nursery diet interaction (S × N) linear ($P < 0.05$); *S × N linear ($P < 0.1$); [#] S × N quadratic ($P < 0.1$).

Table 10. Main effects of *Pichia guilliermondii* (Pg) on concentration and proportions among peripheral blood leukocytes of nursery pigs on d 5 and 28 of the nursery period (Exp. 2).

	Sow Treatment[1]			*P*-value		Sow Treatment	
d 5	SC	S1	S2	Sow	S x N	Linear	Quadratic
WBC (K/uL)[2]	13.59 ± 0.98	14.31 ± 0.98	12.94 ± 0.97	0.61	0.82	0.64	0.39
NE (K/uL)	5.13 ± 0.60	5.77 ± 0.60	5.90 ± 0.59	0.62	0.82	0.37	0.73
LY (K/uL)	7.34 ± 0.56	7.12 ± 0.56	5.86 ± 0.56	0.14	0.75	0.07	0.45
MO (K/uL)	0.60 ± 0.06	0.73 ± 0.06	0.53 ± 0.06	0.08	0.47	0.40	0.04
EO (K/uL)	0.47 ± 0.14	0.66 ± 0.14	0.59 ± 0.14	0.62	0.67	0.54	0.45
BA (K/uL)	0.06 ± 0.01	0.03 ± 0.01	0.07 ± 0.01	0.23	0.41	0.61	0.10
NE:LY	0.75 ± 0.11	0.90 ± 0.11	1.01 ± 0.11	0.26	0.79	0.10	0.91
NE (%)	38.18 ± 2.41	39.99 ± 2.41	44.18 ± 2.40	0.21	0.60	0.09	0.69
LY (%)	53.51 ± 2.65	50.43 ± 2.65	46.97 ± 5.65	0.23	0.83	0.09	0.95
MO (%)	4.44 ± 0.37	5.10 ± 0.37	4.28 ± 0.36	0.25	0.26	0.75	0.11
EO (%)	3.50 ± 0.82	4.25 ± 0.82	4.10 ± 0.82	0.80	0.61	0.61	0.66
BA (%)	0.37 ± 0.07	0.22 ± 0.07	0.48 ± 0.07	0.06	0.26	0.34	0.03
d 28							
WBC (K/uL)	21.88 ± 1.47	23.15 ± 1.47	21.56 ± 1.46	0.72	0.19	0.88	0.43
NE (K/uL)	8.84 ± 0.74	10.40 ± 0.74	8.75 ± 0.74	0.23	0.49	0.93	0.09
LY (K/uL)	10.57 ± 0.94	10.26 ± 0.94	9.99 ± 0.94	0.91	0.14	0.66	0.99
MO (K/uL)	1.06 ± 0.11	0.91 ± 0.11	1.11 ± 0.11	0.42	0.11	0.73	0.20
EO (K/uL)	1.31 ± 0.27	1.52 ± 0.27	1.59 ± 0.27	0.74	0.95	0.46	0.84
BA (K/uL)	0.10 ± 0.02	0.07 ± 0.02	0.12 ± 0.02	0.15	0.11	0.49	0.07
NE:LY	0.90 ± 0.12	1.18 ± 0.12	0.96 ± 0.12	0.22	0.56	0.74	0.09
NE (%)	41.03 ± 2.46	45.61 ± 2.46	40.75 ± 2.45	0.30	0.57	0.94	0.13
LY (%)	47.44 ± 2.35	43.64 ± 2.35	46.33 ± 2.34	0.51	0.46	0.74	0.27
MO (%)	4.85 ± 0.40	4.01 ± 0.40	5.14 ± 0.40	0.13	0.17	0.61	0.05
EO (%)	6.27 ± 1.07	6.45 ± 1.07	7.25 ± 1.07	0.79	0.69	0.52	0.82
BA (%)	0.42 ± 0.06[ab]	0.28 ± 0.06[b]	0.53 ± 0.06[a]	0.02	0.14	0.19	0.02

[1] SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg.

[2] WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/µL) – 1,000 cells/µL; (%) - percentage of total WBC.

Table 10 (cont.). Main effects of *Pichia guilliermondii* (Pg) on concentration and proportions among peripheral blood leukocytes of nursery pigs on d 5 and 28 of the nursery period (Exp. 2).

	Nursery Treatment			*P*-value	
d 5	NC	NPg[1]	SEM	Nursery	S x N
WBC (K/uL)[2]	13.41	13.82	0.80	0.72	0.82
NE (K/uL)	5.47	5.72	0.49	0.73	0.82
LY (K/uL)	6.68	6.86	0.46	0.79	0.75
MO (K/uL)	0.67	0.57	0.05	0.18	0.47
EO (K/uL)	0.52	0.63	0.11	0.49	0.67
BA (K/uL)	0.06	0.05	0.01	0.40	0.41
NE:LY	0.88	0.89	0.09	0.98	0.79
NE (%)	40.82	40.74	1.98	0.98	0.60
LY (%)	50.13	50.48	2.18	0.91	0.83
MO (%)	5.02	4.20	0.30	0.06	0.26
EO (%)	3.62	4.29	0.68	0.49	0.61
BA (%)	0.42	0.30	0.06	0.17	0.26
d 28					
WBC (K/uL)	23.17	21.22	1.20	0.26	0.19
NE (K/uL)	9.88	8.78	0.61	0.21	0.49
LY (K/uL)	10.66	9.89	0.77	0.49	0.14
MO (K/uL)	1.10	0.95	0.09	0.25	0.11
EO (K/uL)	1.41	1.54	0.22	0.68	0.95
BA (K/uL)	0.13	0.07	0.02	0.01	0.11
NE:LY	1.03	0.99	0.10	0.77	0.56
NE (%)	42.86	42.06	2.01	0.78	0.57
LY (%)	45.42	46.18	1.92	0.78	0.46
MO (%)	4.89	4.45	0.33	0.35	0.17
EO (%)	6.32	7.00	0.88	0.59	0.69
BA (%)	0.51	0.31	0.05	0.01	0.14

[1] NC – Nursery control diet; NPg – Nursery control + Pg. Pg added at 0.2% to nursery diet.

[2] WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/µL) – 1,000 cells/µL; (%) - percentage of total WBC.

Table 11. Interactive effects of sow and nursery *Pichia guilliermondii* (Pg) treatments on concentration and proportions among peripheral blood leukocytes of nursery pigs on d 5 and 28 of the nursery period (Exp. 2).

d 5	SC-NC[1]	SC-NPg	S1-NC	S1-NPg	S2-NC	S2-NPg	S x N
WBC (K/uL)[2]	13.83 ± 1.41	13.35 ± 1.39	13.66 ± 1.39	14.97 ± 1.38	12.73 ± 1.39	13.15 ± 1.39	0.82
NE (K/uL)	4.99 ± 0.86	5.26 ± 0.85	5.38 ± 0.85	6.16 ± 0.84	6.05 ± 0.85	5.74 ± 0.85	0.82
LY (K/uL)	7.60 ± 0.81	7.08 ± 0.79	6.84 ± 0.79	7.41 ± 0.79	5.62 ± 0.79	6.10 ± 0.79	0.75
MO (K/uL)	0.65 ± 0.09	0.56 ± 0.09	0.84 ± 0.09	0.62 ± 0.09	0.52 ± 0.09	0.53 ± 0.09	0.47
EO (K/uL)	0.51 ± 0.20	0.43 ± 0.19	0.57 ± 0.19	0.74 ± 0.19	0.46 ± 0.19	0.72 ± 0.19	0.67
BA (K/uL)	0.08 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.41
NE:LY	0.74 ± 0.16	0.76 ± 0.15	0.84 ± 0.15	0.95 ± 0.15	1.06 ± 0.15	0.95 ± 0.15	0.79
NE (%)	36.84 ± 3.48	39.51 ± 3.42	39.47 ± 3.42	40.51 ± 3.40	46.16 ± 3.42	42.20 ± 3.42	0.60
LY (%)	54.10 ± 3.83	52.91 ± 3.77	50.80 ± 3.77	50.06 ± 3.74	45.48 ± 3.77	48.45 ± 3.77	0.83
MO (%)	4.70 ± 0.53	4.19 ± 0.52	6.01 ± 0.52	4.20 ± 0.52	4.34 ± 0.52	4.21 ± 0.52	0.26
EO (%)	3.84 ± 1.19	3.17 ± 1.17	3.54 ± 1.17	4.97 ± 1.16	3.47 ± 1.17	4.73 ± 1.17	0.61
BA (%)	0.52 ± 0.11	0.23 ± 0.10	0.18 ± 0.10	0.25 ± 0.10	0.55 ± 0.10	0.40 ± 0.10	0.26
d 28							
WBC (K/uL)	24.94 ± 2.12	18.81 ± 2.08	22.27 ± 2.08	24.02 ± 2.07	22.30 ± 2.08	20.82 ± 2.08	0.19
NE (K/uL)	10.09 ± 1.07	7.60 ± 1.05	10.79 ± 1.05	10.00 ± 1.05	8.75 ± 1.05	8.75 ± 1.05	0.49
LY (K/uL)[#]	12.22 ± 1.36	8.91 ± 1.34	9.15 ± 1.34	11.38 ± 1.33	10.60 ± 1.34	9.37 ± 1.34	0.14
MO (K/uL)[Q]	1.19 ± 0.16	0.93 ± 0.16	0.79 ± 0.16	1.03 ± 0.16	1.33 ± 0.16	0.89 ± 0.16	0.11
EO (K/uL)	1.28 ± 0.39	1.33 ± 0.38	1.48 ± 0.38	1.56 ± 0.38	1.46 ± 0.38	1.73 ± 0.38	0.95
BA (K/uL)[Q]	0.16 ± 0.03	0.05 ± 0.03	0.06 ± 0.03	0.07 ± 0.03	0.16 ± 0.03	0.08 ± 0.03	0.11
NE:LY	0.88 ± 0.17	0.93 ± 0.17	1.31 ± 0.17	1.06 ± 0.16	0.92 ± 0.17	1.00 ± 0.17	0.56
NE (%)	40.56 ± 3.54	41.49 ± 3.48	48.17 ± 3.48	43.04 ± 3.46	39.84 ± 3.48	41.66 ± 3.48	0.57
LY (%)	48.47 ± 3.39	46.41 ± 3.33	10.86 ± 3.33	46.42 ± 3.31	46.95 ± 3.33	45.71 ± 3.33	0.46
MO (%)	4.88 ± 0.58	4.82 ± 0.57	3.81 ± 0.57	4.22 ± 0.56	5.97 ± 0.57	4.30 ± 0.57	0.17
EO (%)	5.52 ± 1.54	7.02 ± 1.52	6.88 ± 1.52	6.03 ± 1.51	6.56 ± 1.52	7.94 ± 1.52	0.69
BA (%)[Q]	0.57 ± 0.09	0.25 ± 0.09	0.27 ± 0.09	0.28 ± 0.09	0.68 ± 0.09	0.38 ± 0.09	0.14

[1] SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg; NC – Nursery control diet; NPg – Nursery control + Pg. Pg added at 0.2% to nursery diet.

[2] WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/µL) – 1,000 cells/µL; (%) - percentage of total WBC.

[Q]Sow diet × nursery diet interaction (S × N) quadratic ($P < 0.05$); [#]S × N quadratic ($P < 0.1$).

Table 12. Performance of nursery pigs before and after an inflammatory LPS challenge.

	Sow Treatment[1]				*P*-value		Contrasts	
Variable	**SC**	**S1**	**S2**	**SEM**	**Sow**	**Sow x nursery**	**Linear**	**Quadratic**
ADG, g								
-48 to -24 h	132.3[b]	407.8[a]	306.2[ab]	81.5	0.07	0.44	0.14	0.07
-24 to 0 h	287.7	520.3	515.3	107.7	0.24	0.32	0.15	0.38
-48 to 0 h	420.0[b]	928.1[a]	821.5[ab]	159.8	0.08	0.34	0.09	0.13
0 to 24 h	37.4	-170.7	-95.6	106.0	0.39	0.49	0.38	0.29
24 to 48 h	366.7	539.5	593.4	101.2	0.27	0.91	0.12	0.64
0 to 48 h	404.0	368.9	497.7	154.4	0.83	0.66	0.67	0.67
Overall	824.0	1297.0	1319.0	258.3	0.32	0.36	0.19	0.48
ADFI, g								
-48 to -24 h	499	580	631	61	0.31	0.10	0.13	0.85
-24 to 0 h	488[b]	594[ab]	730[a]	71	0.07	0.32	0.02	0.86
-48 to 0 h	987[b]	1173[ab]	1361[a]	120	0.11	0.16	0.04	1.00
0 to 24 h	333	307	389	37	0.29	0.12	0.29	0.25
24 to 48 h	465	540	631	54	0.12	0.68	0.04	0.91
0 to 48 h	798	848	1021	83	0.15	0.36	0.07	0.55
AFIR[2]	84.4	80.7	75.4	6.5	0.62	0.25	0.34	0.92
Gain:feed								
-48 to -24 h	0.168[b]	0.659[a]	0.501[ab]	0.149	0.08	0.46	0.12	0.09
-24 to 0 h	0.320	0.808	0.703	0.264	0.40	0.63	0.31	0.37
-48 to 0 h	0.279[b]	0.757[a]	0.602[ab]	0.153	0.10	0.45	0.15	0.10
0 to 24 h	-0.090	-0.800	-0.653	0.427	0.47	0.31	0.36	0.42
24 to 48 h	0.462	0.926	0.936	0.194	0.16	0.94	0.09	0.35
0 to 48 h	0.246	0.348	0.396	0.219	0.89	0.65	0.63	0.92
Overall	0.294	0.575	0.533	0.141	0.33	0.42	0.24	0.36

Table 12 (cont.). Performance of nursery pigs before and after an inflammatory LPS challenge.

	Sow Treatment[1]				*P*-value		Contrasts	
Variable	**SC**	**S1**	**S2**	**SEM**	**Sow**	**Sow x nursery**	**Linear**	**Quadratic**
Rectal temperature								
-48h	38.80[b]	39.24[a]	39.39[a]	0.14	0.01	0.35	0.005	0.40
-24h	38.74[c]	39.07[b]	39.29[a]	0.08	0.0003	0.25	<0.0001	0.62
0h	38.74[b]	39.27[a]	39.30[a]	0.12	0.005	0.18	0.003	0.12
5 h	39.75[b]	40.17[ab]	40.29[a]	0.15	0.05	0.29	0.02	0.44
24 h	38.68	38.70	38.68	0.12	0.99	0.20	0.98	0.90
48 h	38.49[b]	38.99[a]	38.71[ab]	0.10	0.005	0.85	0.12	0.003
Rectal temperature ratio[3]								
5 h	0.99	0.98	0.96	0.14	0.99	0.35	0.89	0.99
24 h	-0.08[a]	-0.49[b]	-0.65[b]	0.11	0.005	0.10	0.002	0.38
48 h	-0.28[ab]	-0.20[a]	-0.62[b]	0.13	0.06	0.74	0.07	0.12

[1]SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg.

[2]Ratio of feed intake from 0 to 48h/feed intake from -48h to 0h.

[3]Difference of rectal temperature at 5, 24, and 48h post-LPS treatment, respectively and baseline (-48, -24 and 0 h).

[a.b]Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 12 (cont.). Performance of nursery pigs before and after an inflammatory LPS challenge.

	Nursery Treatment			*P*-value			Contrasts	
Variable	**NC**	**NPg[1]**	**SEM**	**Sow**	**Nursery**	**Sow x nursery**	**Linear**	**Quadratic**
ADG, g								
-48 to -24 h	191.8	372.4	67.4	0.07	0.07	0.44	0.14	0.07
-24 to 0 h	447.7	434.5	89.1	0.24	0.92	0.32	0.15	0.38
-48 to 0 h	639.5	806.9	132.2	0.08	0.38	0.34	0.09	0.13
0 to 24 h	-73.5	-79.2	87.8	0.39	0.96	0.49	0.38	0.29
24 to 48 h	532.4	467.4	83.7	0.27	0.59	0.91	0.12	0.64
0 to 48 h	458.8	388.2	127.8	0.83	0.70	0.66	0.67	0.67
Overall	1098.4	1195.1	213.8	0.32	0.75	0.36	0.19	0.48
ADFI, g								
-48 to -24 h	561	579	50	0.31	0.81	0.10	0.13	0.85
-24 to 0 h	582	625	58	0.07	0.61	0.32	0.02	0.86
-48 to 0 h	1143	1204	99	0.11	0.67	0.16	0.04	1.00
0 to 24 h	352	334	31	0.29	0.69	0.12	0.29	0.25
24 to 48 h	587	504	45	0.12	0.20	0.68	0.04	0.91
0 to 48 h	939	838	68	0.15	0.31	0.36	0.07	0.55
AFIR[2]	90.8	69.5	5.4	0.62	0.009	0.25	0.34	0.92
Gain:feed								
-48 to -24 h	0.226	0.659	0.123	0.08	0.02	0.46	0.12	0.09
-24 to 0 h	0.592	0.628	0.218	0.40	0.91	0.63	0.31	0.37
-48 to 0 h	0.442	0.650	0.126	0.10	0.25	0.45	0.15	0.10
0 to 24 h	-0.403	-0.626	0.351	0.47	0.66	0.31	0.36	0.42
24 to 48 h	0.716	0.834	0.161	0.16	0.61	0.94	0.09	0.35
0 to 48 h	0.320	0.340	0.181	0.89	0.94	0.65	0.63	0.92
Overall	0.391	0.543	0.117	0.33	0.37	0.42	0.24	0.36

Table 12 (cont.). Performance of nursery pigs before and after an inflammatory LPS challenge.

	Nursery Treatment			*P*-values			Contrasts	
Variable	**NC**	**NPg**	**SEM**	**Sow**	**Nursery**	**Sow x Nursery**	**Linear**	**Quadratic**
Rectal temperature								
-48h	39.16	39.13	0.11	0.01	0.82	0.35	0.005	0.40
-24h	39.07	39.00	0.07	0.0003	0.52	0.25	<0.0001	0.62
0h	39.01	39.20	0.10	0.005	0.19	0.18	0.003	0.12
5 h	39.98	40.16	0.13	0.05	0.32	0.29	0.02	0.44
24 h	38.59	37.78	0.11	0.99	0.23	0.20	0.98	0.90
48 h	38.78	38.68	0.08	0.005	0.40	0.85	0.12	0.003
Rectal temperature ratio[3]								
5 h	0.90	1.05	0.12	0.99	0.37	0.35	0.89	0.99
24 h	-0.49	-0.33	0.09	0.005	0.26	0.10	0.002	0.38
48 h	-0.30	-0.43	0.10	0.06	0.39	0.74	0.07	0.12

[1]NC – Nursery control diet; NPg – Nursery control + Pg. Pg added at 0.2% to nursery diet.
[2]Ratio of feed intake from 0 to 48h/feed intake from -48h to 0h.
[3]Difference of rectal temperature at 5, 24, and 48h post-LPS treatment, respectively and baseline (-48, -24 and 0 h).
[a.b]Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 13. Main effects of LPS challenge on serum IL-1β, IL-6, BUN, CRP, SAA, and haptoglobin concentrations of the nursery pig.

	Sow Treatment[1]			Nursery Treatment	
Item[2]	**SC**	**S1**	**S2**	**NC**	**NPg**
IL-1β, pg/mL	104.19 ± 14.37^{ab}	140.71 ± 14.37^{a}	94.68 ± 14.37^{b}	108.76 ± 11.73	117.62 ± 11.73
IL-6, pg/mL	165.95 ± 61.86^{ab}	468.38 ± 61.86^{a}	259.23 ± 61.86^{b}	310.16 ± 50.51	285.55 ± 50.51
BUN, mg/dL	12.93 ± 1.26	12.10 ± 1.26	11.29 ± 1.26	13.81 ± 1.14^{a}	10.39 ± 1.14^{b}
CRP, µg/mL	137.17 ± 37.92	204.22 ± 37.47	225.18 ± 36.17	168.96 ± 30.93	208.75 ± 29.80
SAA, µg/mL	96.07 ± 16.89	121.77 ± 17.99	126.31 ± 17.51	122.03 ± 14.40	107.41 ± 14.13
Haptoglobin, mg/mL	4.21 ± 0.42	3.23 ± 0.42	3.04 ± 0.42	3.47 ± 0.34	3.51 ± 0.34

	Time			*P*-value[3]				
	0 h	**5 h**	**24 h**	**Sex**	**Time**	**ST**	**NT**	**ST x NT**
IL-1β	76.39 ± 7.64^{b}	199.98 ± 23.67^{a}	63.20 ± 0.68^{b}	0.55	< 0.0001	0.06	0.59	0.13
IL-6	131.62 ± 3.68^{b}	631.50 ±105.83^{a}	130.45 ± 3.72^{b}	0.62	< 0.0001	0.003	0.73	0.62
BUN	10.62 ± 1.10^{b}	12.46 ± 1.10^{a}	13.23 ± 1.10^{a}	0.002	0.01	0.47	0.003	0.005
CRP	135.29 ± 32.82^{b}	60.22 ± 4.43^{c}	371.06 ± 55.25^{a}	0.05	< 0.0001	0.23	0.36	0.24
SAA	1.98 ± 0.46^{c}	52.22 ± 5.24^{b}	289.96 ± 27.79^{a}	0.48	< 0.0001	0.40	0.47	0.28
Haptoglobin	3.15 ± 0.28^{b}	2.87 ± 0.25^{c}	4.46 ± 0.24^{a}	0.34	< 0.0001	0.11	0.93	0.54

	Contrasts					
	L T	**Q T**	**L ST**	**Q ST**	**L ST X NT**	**Q ST X NT**
IL-1β	< 0.0001	< 0.0001	0.64	0.02	0.14	0.15
IL-6	< 0.0001	< 0.0001	0.29	0.001	0.98	0.33
BUN	0.01	0.05	0.22	0.99	0.001	0.50
CRP	< 0.0001	< 0.0001	0.10	0.62	0.34	0.17
SAA	< 0.0001	0.12	0.22	0.63	0.47	0.15
Haptoglobin	< 0.0001	< 0.0001	0.05	0.45	0.55	0.35

[1] SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg; NC – Nursery control diet; NPg – Nursery control + Pg. Pg added at 0.2% to nursery diet.

[2] BUN – Blood urea nitrogen; CRP – C-reactive protein; SAA – Serum amyloid A.

[3] T – time; ST – sow treatment; NT – nursery treatment; L – linear contrast; Q – quadratic contrast.

[abcd] Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 14. Interactive effects of time by sow treatment on serum IL-1β, IL-6, BUN, CRP, SAA, and haptoglobin concentration of the LPS-challenged nursery pig.

	0 h			5 h			24 h			Time
Item[1]	**SC**[2]	**S1**	**S2**	**SC**	**S1**	**S2**	**SC**	**S1**	**S2**	**x ST**
IL-1β[3]	101.5 ± 13.2^{bc}	62.8 ± 13.2^{d}	64.8 ± 13.2^{cd}	146.5 ± 41.0^{bc}	296.7 ± 41.0^{a}	156.7 ± 41.0^{b}	64.5 ± 1.2^{d}	62.6 ± 1.2^{d}	62.5 ± 1.2^{d}	0.01
IL-6[3]	125.0 ± 6.4^{d}	124.7 ± 6.4^{d}	145.2 ± 6.4^{c}	246.4 ± 183.3^{bcd}	1155.8 ± 183.3^{a}	492.3 ± 183.3^{bc}	126.4 ± 6.5^{d}	124.7 ± 6.5^{d}	140.2 ± 6.5^{bd}	0.002
BUN[4]	13.1 ± 1.5^{a}	9.7 ± 1.5^{bc}	9.0 ± 1.5^{c}	13.7 ± 1.5^{a}	12.3 ± 1.5^{ab}	11.4 ± 1.5^{abc}	12.0 ± 1.5^{abc}	14.3 ± 1.5^{a}	13.4 ± 1.5^{a}	0.07
CRP[5]	72.9 ± 58.6	99.7 ± 55.9	233.3 ± 55.9	55.5 ± 7.5	55.8 ± 7.6	69.3 ± 7.9	283.1 ± 97.2	457.1 ± 97.2	373.0 ± 92.7	0.26
SAA[5]	2.5 ± 0.7	1.9 ± 0.7	1.5 ± 0.7	46.9 ± 8.6	46.1 ± 8.9	63.7 ± 9.7	238.9 ± 46.3	317.3 ± 49.9	313.7 ± 48.1	0.39
Haptoglobin[6]	4.0 ± 0.5	2.8 ± 0.5	2.6 ± 0.5	3.6 ± 0.4	2.6 ± 0.4	2.5 ± 0.4	5.0 ± 0.4	4.3 ± 0.4	4.0 ± 0.4	0.60

[1] BUN – Blood urea nitrogen; CRP – C-reactive protein; SAA – Serum amyloid A; T x ST – time × sow diet interaction.
[2] SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg.
[3] pg/mL.
[4] mg/dL.
[5] µg/mL.
[6] mg/mL.
[abc] Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 15. Interactive effect of time by nursery treatment on serum IL-1β, IL-6, BUN, CRP, SAA, and haptoglobin concentrations of the LPS-challenged nursery pig.

	0 h		5 h		24 h		T x
Item[1]	**NC**	**NPg**[2]	**NC**	**NPg**	**NC**	**NPg**	**NT**
IL-1β[3]	82.4 ± 10.8	70.4 ± 10.8	180.1 ± 33.5	219.8 ± 33.5	63.8 ± 1.0	62.6 ± 1.0	0.54
IL-6[3]	133.4 ± 5.3	129.9 ± 5.26	667.6 ± 149.7	595.4 ± 149.7	129.5 ± 5.3	131.4 ± 5.3	0.12
BUN[4]	13.3 ± 1.3^{ab}	8.0 ± 1.3^{c}	14.4 ± 1.3^{a}	10.5 ± 1.3^{b}	13.8 ± 1.3^{a}	12.7 ± 1.3^{a}	0.05
CRP[5]	97.0 ± 45.7	173.5 ± 47.2	60.4 ± 6.3	60.1 ± 6.2	349.5 ± 80.5	392.6 ± 75.7	0.48
SAA[5]	2.0 ± 0.6	2.00 ± 0.6	49.6 ± 7.1	54.9 ± 7.7	314.5 ± 39.8	265.4 ± 38.8	0.45
Haptoglobin[6]	3.0 ± 0.4	3.3 ± 0.4	3.0 ± 0.3	2.8 ± 0.3	4.4 ± 0.3	4.5 ± 0.3	0.06

[1] BUN – Blood urea nitrogen; CRP – C-reactive protein; SAA – Serum amyloid A; T x NT – time × nursery diet interaction.
[2] Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3).
[3] pg/mL.
[4] mg/dL.
[5] µg/mL.
[6] mg/mL.
[abc] Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 16. Main effects of LPS challenge on concentration and proportions among peripheral blood leukocytes of the nursery pig.

Item[1]	Sow Treatment[2]			Nursery Treatment	
	SC	S1	S2	NC	NPg[2]
WBC (K/uL)	23.61 ± 1.82	18.90 ± 1.82	22.59 ± 1.82	21.43 ± 1.62	21.97 ± 1.63
NE (K/uL)	11.45 ± 1.12	8.85 ± 1.12	9.64 ± 1.12	9.91 ± 0.97	10.05 ± 0.98
LY (K/uL)	9.46 ± 0.87	8.19 ± 0.87	10.57 ± 0.87	9.22 ± 0.74	9.59 ± 0.74
MO (K/uL)	0.57 ± 0.05^{b}	0.54 ± 0.05^{b}	0.76 ± 0.05^{a}	0.58 ± 0.04	0.66 ± 0.04
EO (K/uL)	2.00 ± 0.33	1.37 ± 0.33	1.52 ± 0.33	1.52 ± 0.28	1.74 ± 0.28
BA (K/uL)	0.13 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.11 ± 0.02
NE:LY	1.65 ± 0.19^{a}	1.27 ± 0.19^{ab}	1.05 ± 0.19^{b}	1.36 ± 0.15	1.29 ± 0.15
NE (%)	48.86 ± 2.63	45.20 ± 2.64	42.54 ± 2.63	46.08 ± 2.16	44.99 ± 2.17
LY (%)	39.96 ± 2.61	42.44 ± 2.62	45.65 ± 2.61	43.10 ± 2.18	42.26 ± 2.19
MO (%)	2.41 ± 0.21	2.73 ± 0.21	3.20 ± 0.21	2.66 ± 0.17	2.90 ± 0.18
EO (%)	8.29 ± 1.42	9.18 ± 1.42	8.17 ± 1.42	7.68 ± 1.16	9.41 ± 1.16
BA (%)	0.49 ± 0.08	0.53 ± 0.08	0.44 ± 0.08	0.43 ± 0.07	0.54 ± 0.07

	Time		
	0 h	5 h	24 h
WBC (K/uL)	24.71 ± 1.41^{a}	15.91 ± 1.99^{b}	24.47 ± 1.59^{a}
NE (K/uL)	10.17 ± 0.76	8.76 ± 1.32	11.01 ± 0.87
LY (K/uL)	11.59 ± 0.71^{a}	4.66 ± 0.56^{b}	11.98 ± 0.67^{a}
MO (K/uL)	0.95 ± 0.07^{a}	0.24 ± 0.02^{c}	0.68 ± 0.04^{b}
EO (K/uL)	1.93 ± 0.32^{a}	2.20 ± 0.31^{a}	0.78 ± 0.18^{b}
BA (K/uL)	0.13 ± 0.02^{a}	0.10 ± 0.02^{ab}	0.08 ± 0.02^{b}
NE:LY	0.98 ± 0.08^{b}	2.01 ± 0.22^{a}	0.98 ± 0.07^{b}
NE (%)	41.67 ± 1.72^{c}	50.10 ± 2.31^{a}	44.82 ± 1.41^{b}
LY (%)	46.42 ± 1.69^{b}	32.43 ± 2.32^{c}	49.20 ± 1.67^{a}
MO (%)	3.85 ± 0.24	1.71 ± 0.10	2.78 ± 0.12
EO (%)	7.58 ± 0.96^{b}	15.13 ± 1.56^{a}	2.94 ± 0.40^{c}
BA (%)	0.50 ± 0.06^{a}	0.66 ± 0.08^{a}	0.29 ± 0.03^{b}

Table 16 (cont.) Main effects of LPS challenge on concentration and proportions among peripheral blood leukocytes of the nursery pig.

	P-value[3]					Contrasts					
	Sex	**Time**	**ST**	**NT**	**ST x NT**	**L T**	**Q T**	**L ST**	**Q ST**	**L ST X NT**	**Q ST X NT**
WBC (K/uL)	0.43	< 0.0001	0.52	0.75	0.52	0.05	< 0.0001	0.61	0.02	0.88	0.26
NE (K/uL)	0.82	0.28	0.14	0.89	0.19	0.15	0.13	0.18	0.15	0.42	0.10
LY (K/uL)	0.95	< 0.0001	0.12	0.69	0.38	< 0.0001	< 0.0001	0.33	0.07	0.19	0.68
MO (K/uL)	0.20	< 0.0001	0.01	0.19	0.39	0.28	< 0.0001	0.01	0.06	0.21	0.59
EO (K/uL)	0.36	< 0.0001	0.32	0.54	0.90	< 0.0001	0.14	0.27	0.30	0.66	0.93
BA (K/uL)	0.42	0.002	0.39	0.40	0.62	0.0004	0.55	0.26	0.43	0.99	0.33
NE:LY	0.08	< 0.0001	0.07	0.72	0.47	0.0016	< 0.0001	0.03	0.70	0.61	0.26
NE (%)	0.61	< 0.0001	0.24	0.72	0.38	0.80	< 0.0001	0.09	0.88	0.40	0.27
LY (%)	0.44	< 0.0001	0.28	0.78	0.33	< 0.0001	< 0.0001	0.11	0.91	0.21	0.42
MO (%)	0.34	< 0.0001	0.03	0.32	0.90	0.03	< 0.0001	0.01	0.77	0.64	0.99
EO (%)	0.05	< 0.0001	0.86	0.30	0.66	< 0.0001	< 0.0001	0.96	0.59	0.47	0.58
BA (%)	0.14	< 0.0001	0.77	0.23	0.69	< 0.0001	0.01	0.70	0.54	0.61	0.49

[1] WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/µL) – 1,000 cells/µL; (%) - percentage of total WBC.

[2] SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg; NC – Nursery control diet; NPg – Nursery control + Pg. Pg added at 0.2% to nursery diet.

[3] T – time; ST – sow treatment; NT – nursery treatment; L – linear contrast; Q – quadratic contrast.

[abc] Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 17. Interactive effect of time by sow treatment on concentration and proportions among peripheral blood leukocytes of the LPS-challenged nursery pig.

	0 h			5 h			24 h			
Item[1]	**SC**[2]	**S1**	**S2**	**SC**	**S1**	**S2**	**SC**	**S1**	**S2**	T x ST
WBC (K/uL)	25.2 ± 1.8	21.7 ± 1.8	27.3 ± 1.8	21.9 ± 3.0	11.6 ± 3.0	14.3 ± 3.0	23.8 ± 2.2	23.4 ± 2.2	23.2 ± 2.2	0.08
NE (K/uL)	10.3 ± 1.0^{a}	9.7 ± 1.0^{a}	10.5 ± 1.0^{a}	13.0 ± 2.1^{a}	6.0 ± 2.1^{b}	7.3 ± 2.1^{a}	11.1 ± 1.3^{a}	10.8 ± 1.3^{a}	11.1 ± 1.3^{a}	0.05
LY (K/uL)	11.8 ± 1.1	9.6 ± 1.1	13.4 ± 1.1	5.3 ± 0.8	3.7 ± 0.8	5.0 ± 0.8	11.3 ± 1.0	11.3 ± 1.0	13.3 ± 1.0	0.17
MO (K/uL)	0.8 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	0.30 ± 0.03	0.18 ± 0.03	0.25 ± 0.03	0.62 ± 0.07	0.59 ± 0.07	0.82 ± 0.07	0.09
EO (K/uL)	2.2 ± 0.5	1.5 ± 0.5	2.0 ± 0.5	3.2 ± 0.5	1.8 ± 0.5	1.6 ± 0.5	0.6 ± 0.3	0.8 ± 0.3	0.9 ± 0.3	0.04
BA (K/uL)	0.16 ± 0.04	0.11 ± 0.04	0.12 ± 0.04	0.15 ± 0.03	0.10 ± 0.03	0.07 ± 0.03	0.08 ± 0.02	0.06 ± 0.02	0.09 ± 0.02	0.24
NE:LY	1.0 ± 0.1^{bcd}	1.1 ± 0.1^{bc}	0.8 ± 0.1^{d}	2.9 ± 0.4^{a}	1.7 ± 0.4^{b}	1.5 ± 0.4^{bc}	1.1 ± 0.1^{bcd}	1.0 ± 0.1^{bcd}	0.9 ± 0.1^{cd}	0.001
NE (%)	41.8 ± 3.0^{cd}	44.5 ± 3.0^{bcd}	38.7 ± 3.0^{d}	57.8 ± 4.0^{a}	45.68 ± 4.01^{bcd}	46.8 ± 4.0^{bc}	47.0 ± 2.4^{b}	45.4 ± 2.5^{bcd}	42.1 ± 2.4^{bcd}	0.01
LY (%)	46.4 ± 2.7	44.1 ± 2.7	48.8 ± 2.7	25.7 ± 3.9	34.49 ± 3.90	37.1 ± 3.9	47.8 ± 2.7	48.8 ± 2.7	51.0 ± 2.7	0.11
MO (%)	3.1 ± 0.4	3.9 ± 0.4	4.5 ± 0.4	1.5 ± 0.2	1.7 ± 0.2	2.0 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	3.1 ± 0.2	0.35
EO (%)	8.1 ± 1.7	7.1 ± 1.7	7.5 ± 1.7	14.5 ± 2.7	17.4 ± 2.7	13.5 ± 2.7	2.2 ± 0.7	3.1 ± 0.7	3.5 ± 0.7	0.50
BA (%)	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.29 ± 0.06	0.26 ± 0.06	0.32 ± 0.06	0.23

[1] WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/µL) – 1,000 cells/µL; (%) - percentage of total WBC.

[2] SC - Sow control diet; S1 – Sow control diet + 0.1% Pg; S2 - Sow control diet + 0.2% Pg.

[abcd] Means in the same row (within main effects) with different superscripts are different ($P < 0.05$).

Table 18. Interactive effect of time by nursery treatment on concentration and proportions among peripheral blood leukocytes of the LPS-challenged nursery pig.

	0 h		5 h		24 h		
Item[1]	NC	NPg[2]	NC	NPg	NC	NPg	T x NT
WBC (K/uL)	24.53 ± 1.65	24.89 ± 1.65	16.43 ± 2.58	15.39 ± 2.58	23.32 ± 1.95	25.62 ± 1.95	0.67
NE (K/uL)	10.37 ± 0.90	9.97 ± 0.90	8.93 ± 1.78	8.59 ± 1.78	10.41 ± 1.09	11.61 ± 1.09	0.52
LY (K/uL)	11.30 ± 0.95	11.87 ± 0.96	4.86 ± 0.72	4.46 ± 0.72	11.51 ± 0.88	12.44 ± 0.88	0.43
MO (K/uL)	0.87 ± 0.09	1.04 ± 0.09	0.25 ± 0.03	0.24 ± 0.03	0.64 ± 0.06	0.72 ± 0.06	0.44
EO (K/uL)	1.78 ± 0.43	2.07 ± 0.43	2.19 ± 0.43	2.20 ± 0.43	0.60 ± 0.23	0.95 ± 0.23	0.83
BA (K/uL)	0.12 ± 0.03	0.14 ± 0.03	0.10 ± 0.03	0.11 ± 0.03	0.06 ± 0.02	0.09 ± 0.02	0.77
NE:LY	1.06 ± 0.11	0.91 ± 0.11	2.04 ± 0.31	1.97 ± 0.31	0.99 ± 0.10	0.98 ± 0.10	0.40
NE (%)	43.02 ± 2.44	40.33 ± 2.46	50.00 ± 3.27	50.21 ± 3.29	45.22 ± 2.00	44.42 ± 2.02	0.59
LY (%)	46.07 ± 2.27	46.78 ± 2.29	33.96 ± 3.21	30.89 ± 3.22	49.28 ± 2.24	49.12 ± 2.26	0.57
MO (%)	3.52 ± 0.34	4.17 ± 0.34	1.72 ± 0.14	1.70 ± 0.14	2.72 ± 0.17	2.84 ± 0.17	0.26
EO (%)	6.86 ± 1.35	8.30 ± 1.36	13.72 ± 2.21	16.53 ± 2.21	2.47 ± 0.56	3.40 ± 0.57	0.77
BA (%)	0.48 ± 0.09	0.53 ± 0.09	0.55 ± 0.12	0.78 ± 0.12	0.25 ± 0.05	0.32 ± 0.05	0.53

[1] WBC – White blood cells; NE – Neutrophils; LY – Lymphocytes; MO – Monocytes; EO – Eosinophils; BA – Basophils; NE:LY - Neutrophil:lymphocyte ratio; (K/µL) – 1,000 cells/µL; (%) - percentage of total WBC.

[2] NC – Nursery control diet; NPg – Nursery control + Pg. Pg added at 0.2% in the nursery diet.

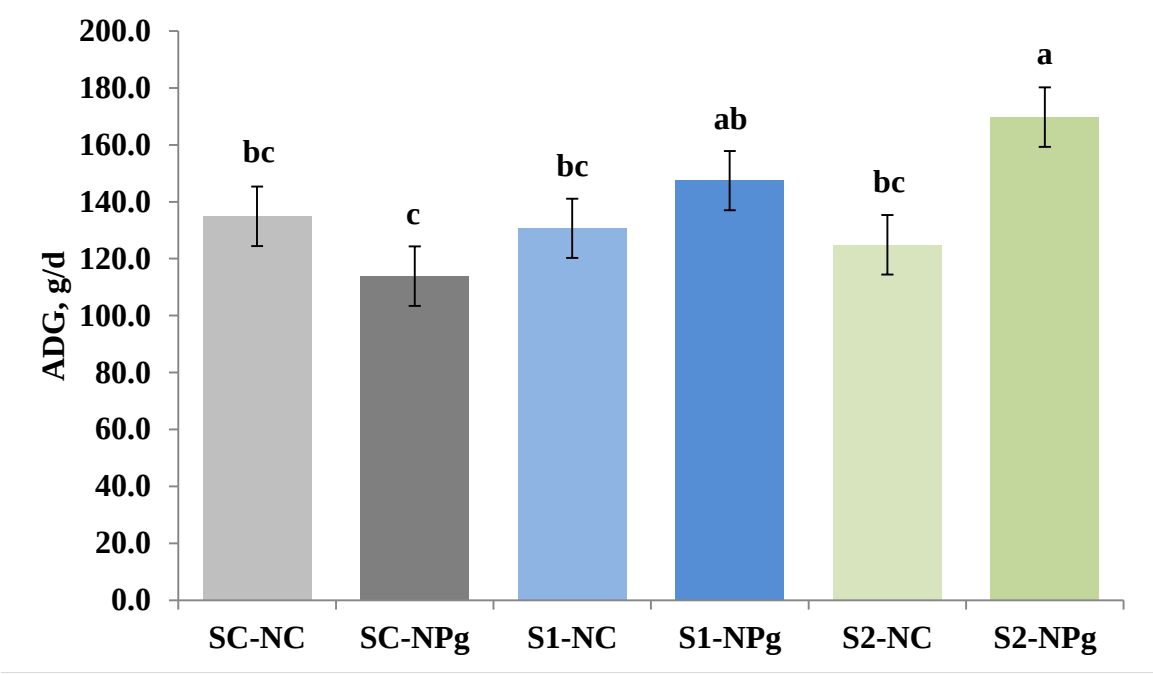

Figure 1. Effect of *Pichia guilliermondii* (Pg) fed in sow and nursery diets on average daily gain during phase 1, Exp. 1 (sow diet x nursery diet, *P* = 0.01). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg; NC – Nursery control; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) in the nursery diet. Bars with different letters differ ($P < 0.05$).

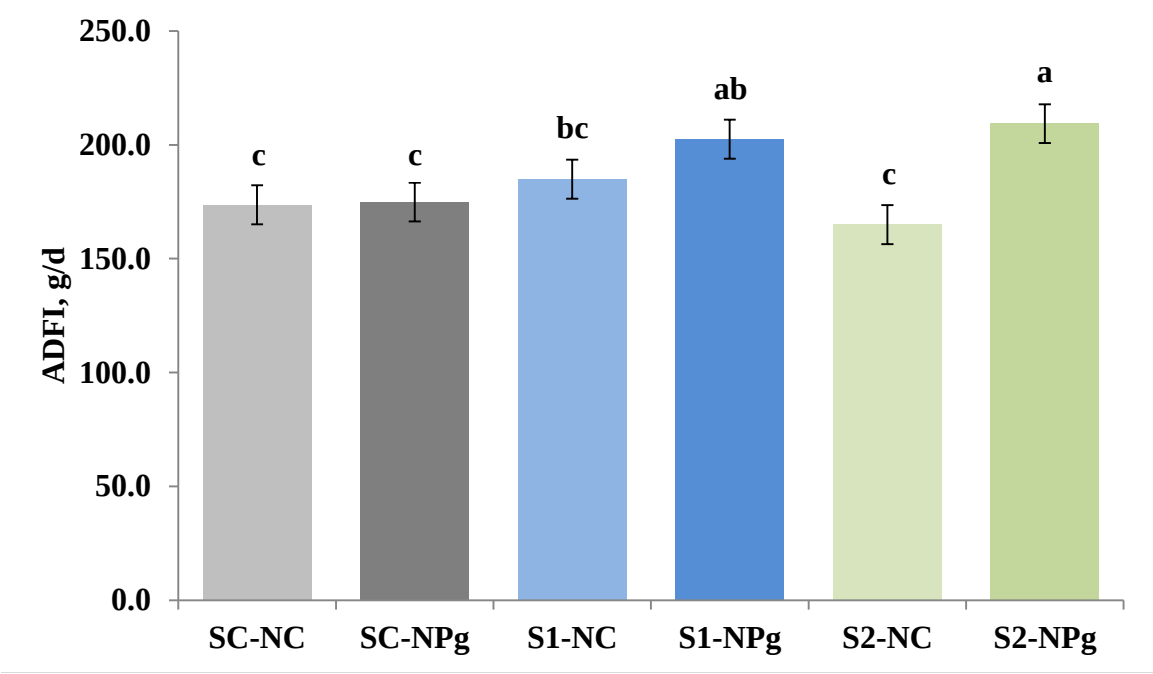

Figure 2. Effect of *Pichia guilliermondii* (Pg) fed in sow and nursery diets on average daily feed intake during phase 1, Exp. 1 (sow diet x nursery diet, *P* = 0.05). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg; NC – Nursery control; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) in the nursery diet. Bars with different letters differ ($P < 0.05$).

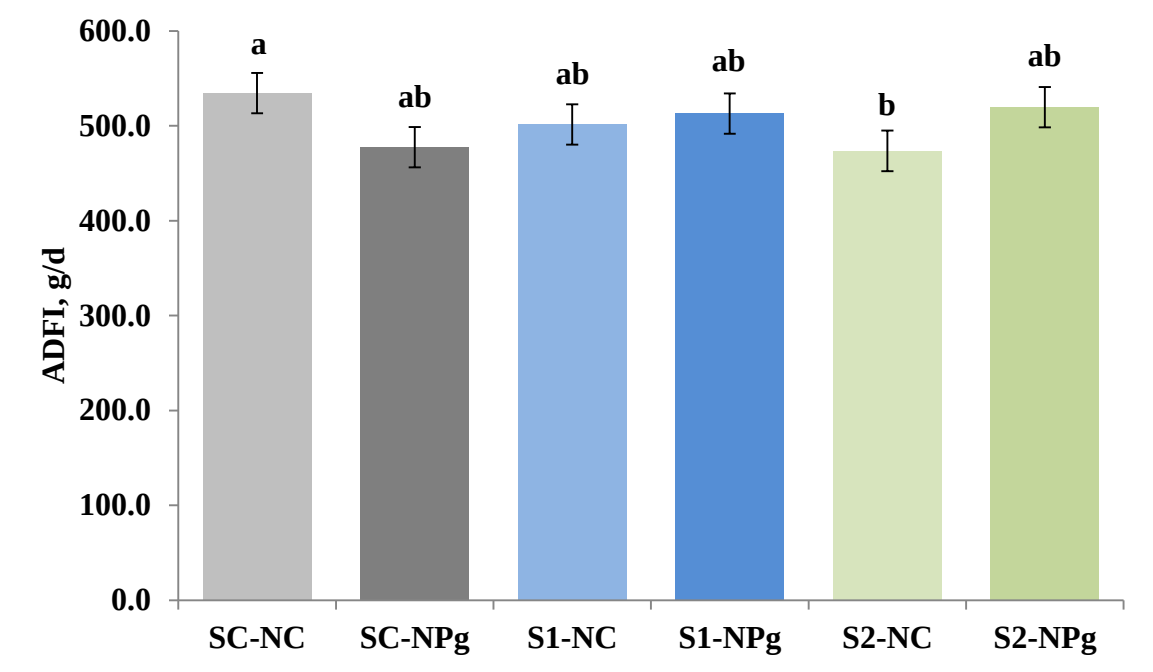

Figure 3. Effect of *Pichia guilliermondii* (Pg) fed in sow and nursery diets on average daily feed intake during Phase 2, Exp. 1 (sow diet x nursery diet, *P* = 0.06). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg; NC – Nursery control; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) in the nursery diet. Bars with different letters differ ($P < 0.05$).

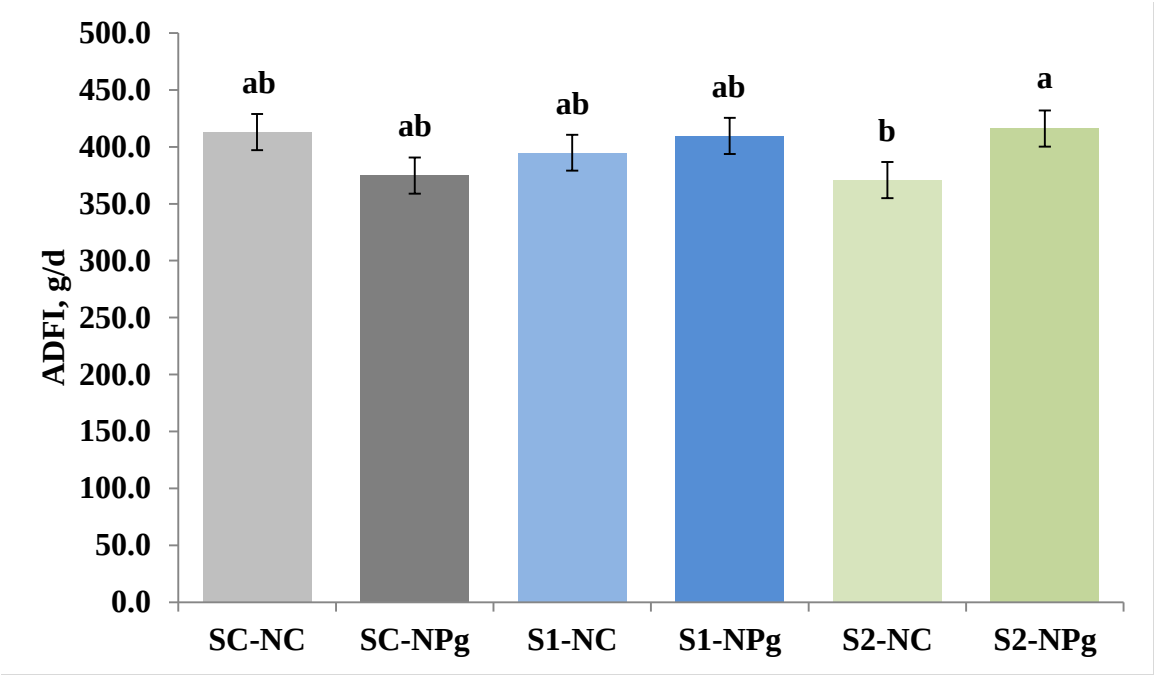

Figure 4. Effect of *Pichia guilliermondii* (Pg) fed in sow and nursery diets on average daily feed intake during the combined phase 1 and 2 period, Exp. 1 (sow diet x nursery diet, *P* = 0.04). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg; NC – Nursery control; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) in the nursery diet. Bars with different letters differ ($P < 0.05$).

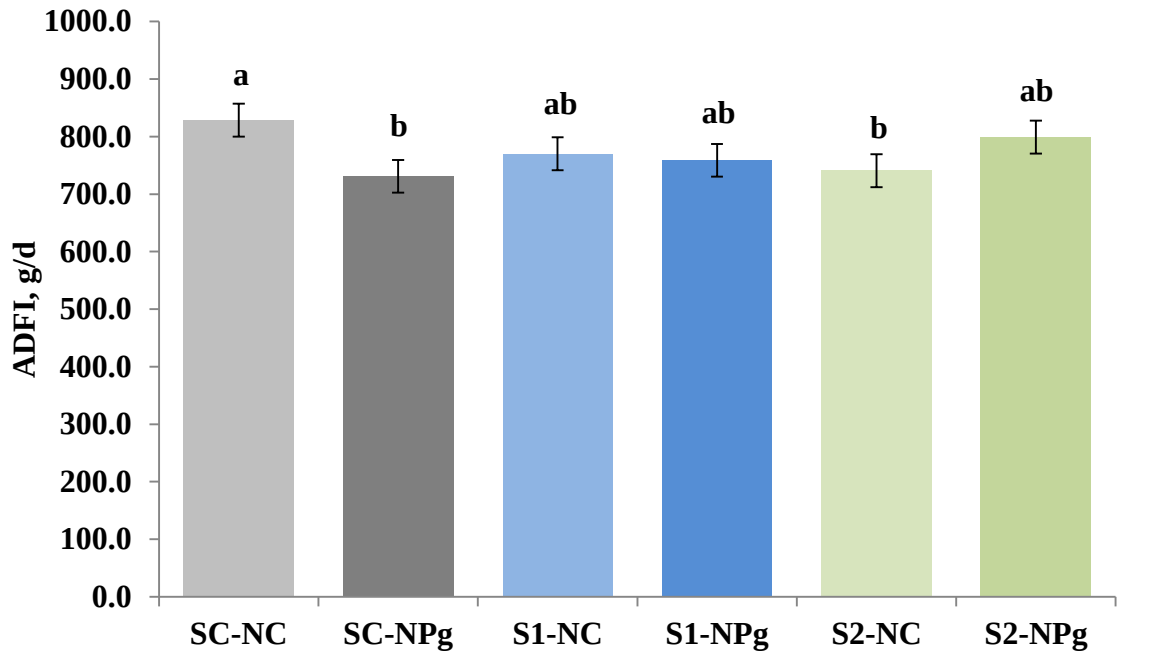

Figure 5. Effect of *Pichia guilliermondii* (Pg) fed in sow and nursery diets on average daily feed intake during phase 3, Exp. 1 (sow diet x nursery diet, *P* = 0.03). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg; NC – Nursery control; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) in the nursery diet. Bars with different letters differ ($P < 0.05$).

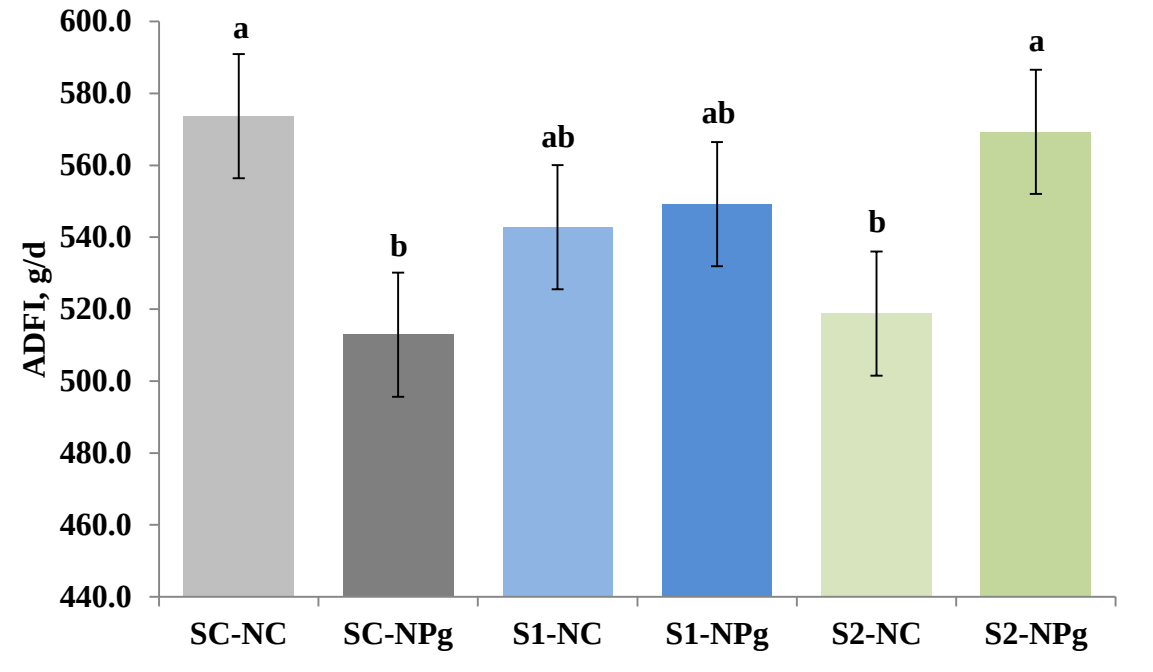

Figure 6. Effect of *Pichia guilliermondii* (Pg) fed in sow and nursery diets on average daily feed intake during the overall nursery period, Exp. 1 (sow diet x nursery diet, *P* = 0.01). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg; NC – Nursery control; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) in the nursery diet. Bars with different letters differ ($P < 0.05$).

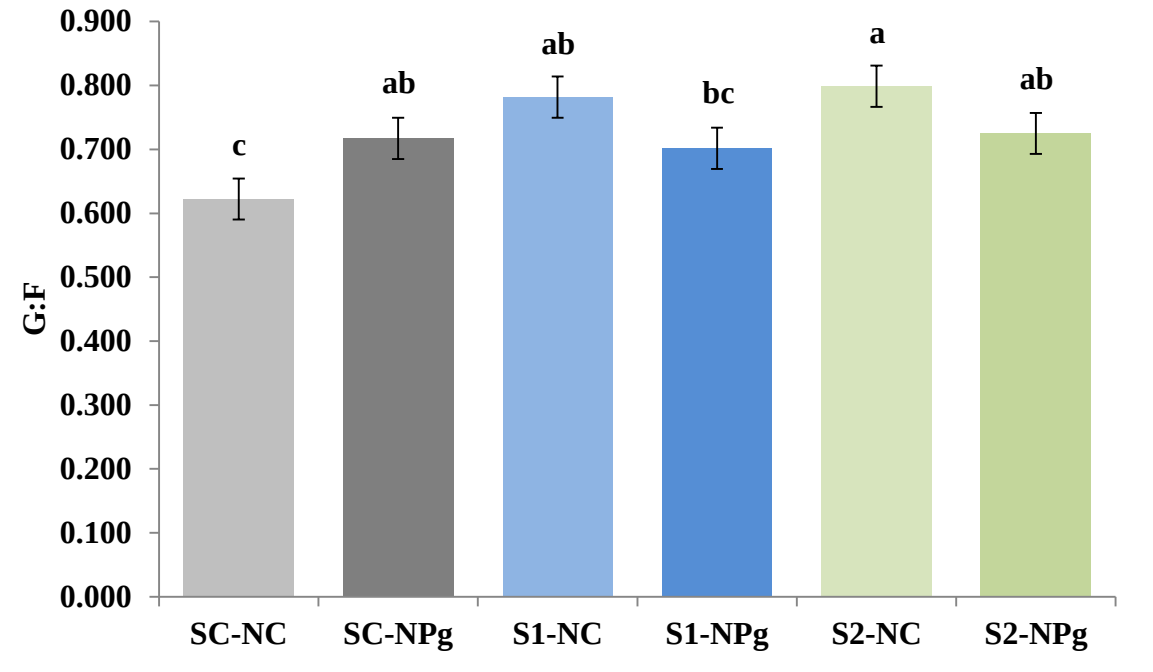

Figure 7. Effect of *Pichia guilliermondii* (Pg) fed in sow and nursery diets on feed efficiency during phase 2, Exp. 1 (sow diet x nursery diet, *P* = 0.01). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg; NC – Nursery control; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) in the nursery diet. Bars with different letters differ ($P < 0.05$).

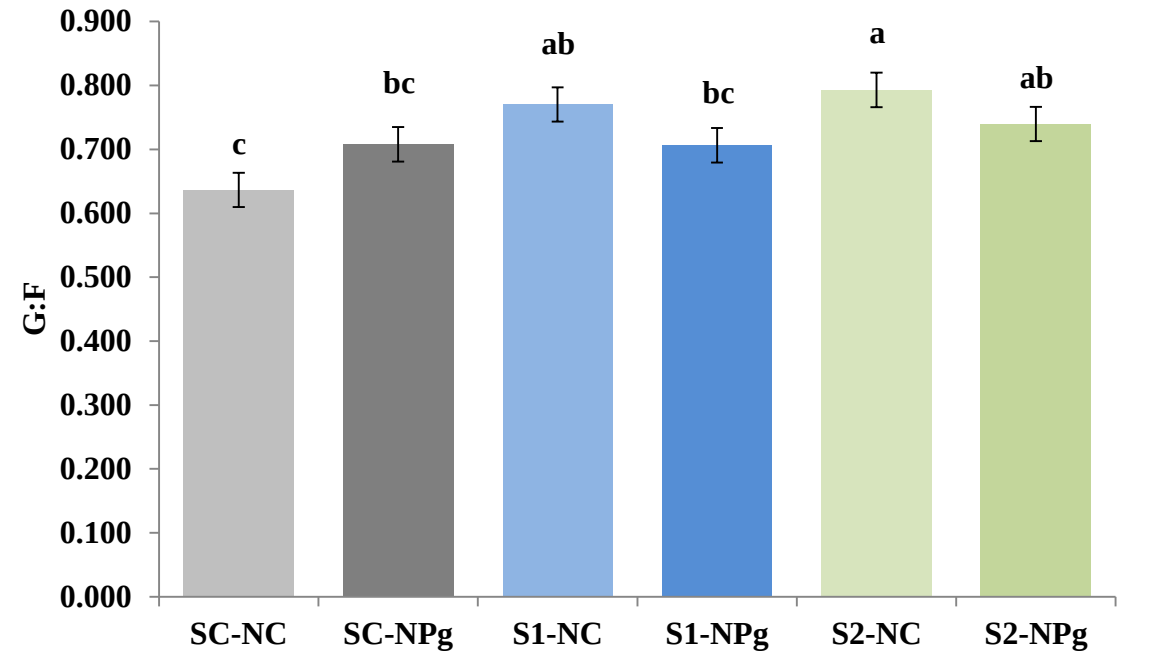

Figure 8. Effect of *Pichia guilliermondii* (Pg) fed in sow and nursery diets on feed efficiency during the combined phase 1 and 2 period, Exp. 1 (sow diet x nursery diet, *P* = 0.03). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg; NC – Nursery control; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) in the nursery diet. Bars with different letters differ ($P < 0.05$).

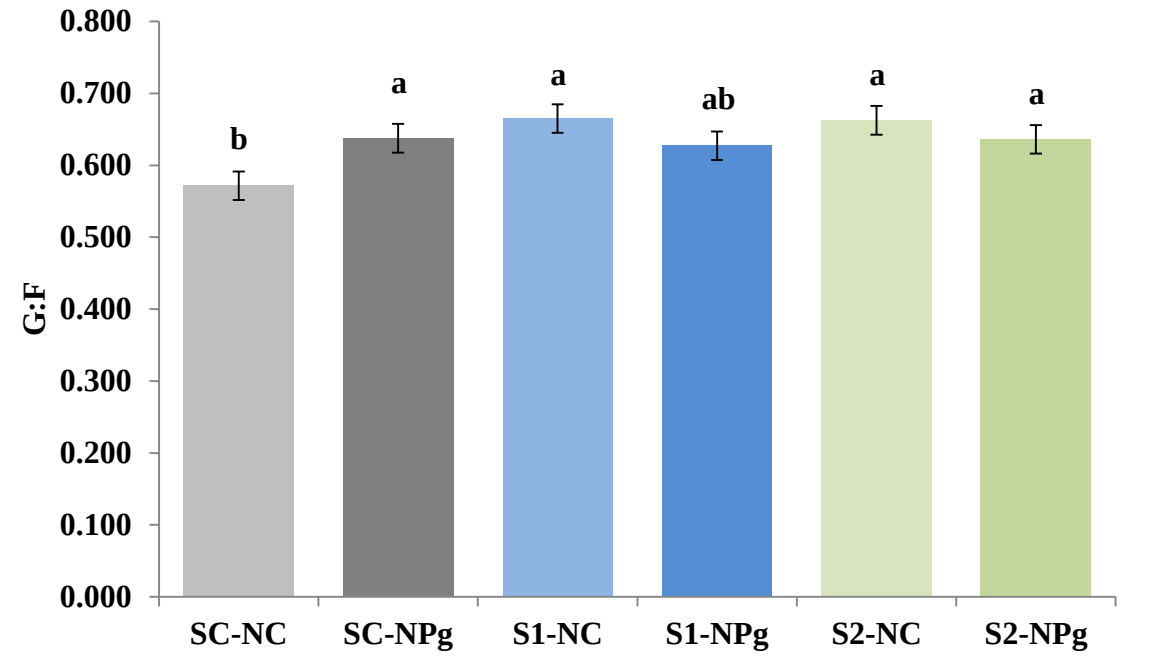

Figure 9. Effect of *Pichia guilliermondii* (Pg) fed in sow and nursery diets on feed efficiency during the overall nursery period, Exp. 1 (sow diet x nursery diet, *P* = 0.02). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg; NC – Nursery control; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) in the nursery diet. Bars with different letters differ ($P < 0.05$).

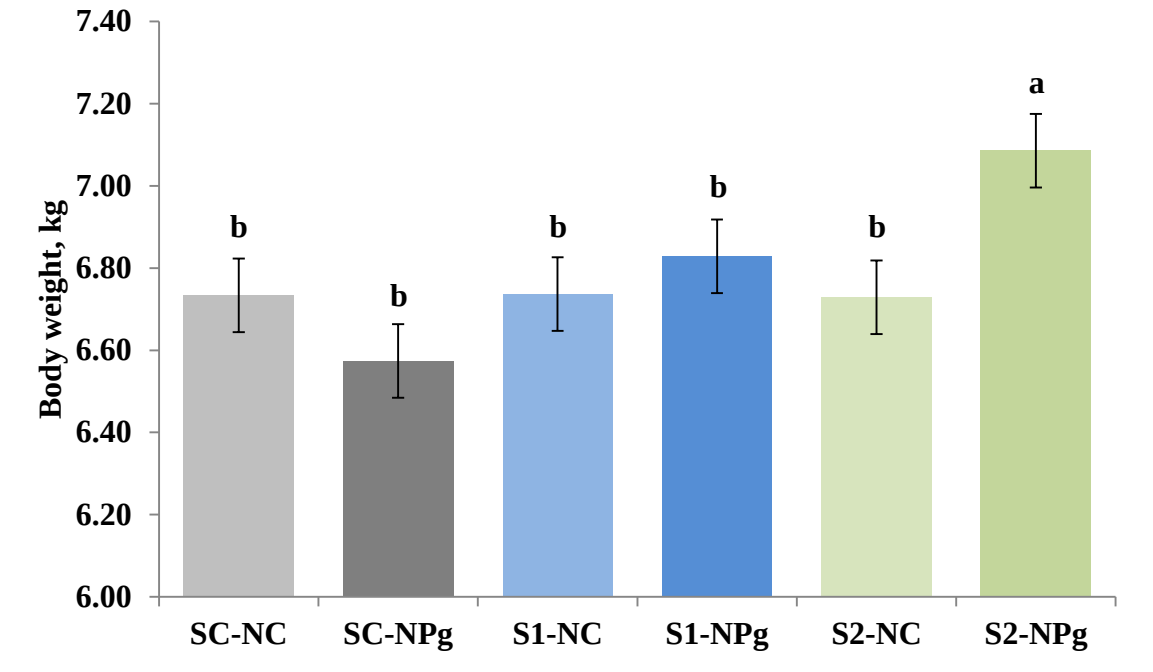

Figure 10. Effect of *Pichia guilliermondii* (Pg) fed in sow and nursery diets on body weight during phase 1, Exp. 1 (sow diet x nursery diet, *P* = 0.02). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg; NC – Nursery control; NPg – Nursery control + Pg. Pg added at 0.2% (Phase 1), and 0.1% (Phase 2 and 3) in the nursery diet. Bars with different letters differ ($P < 0.05$).

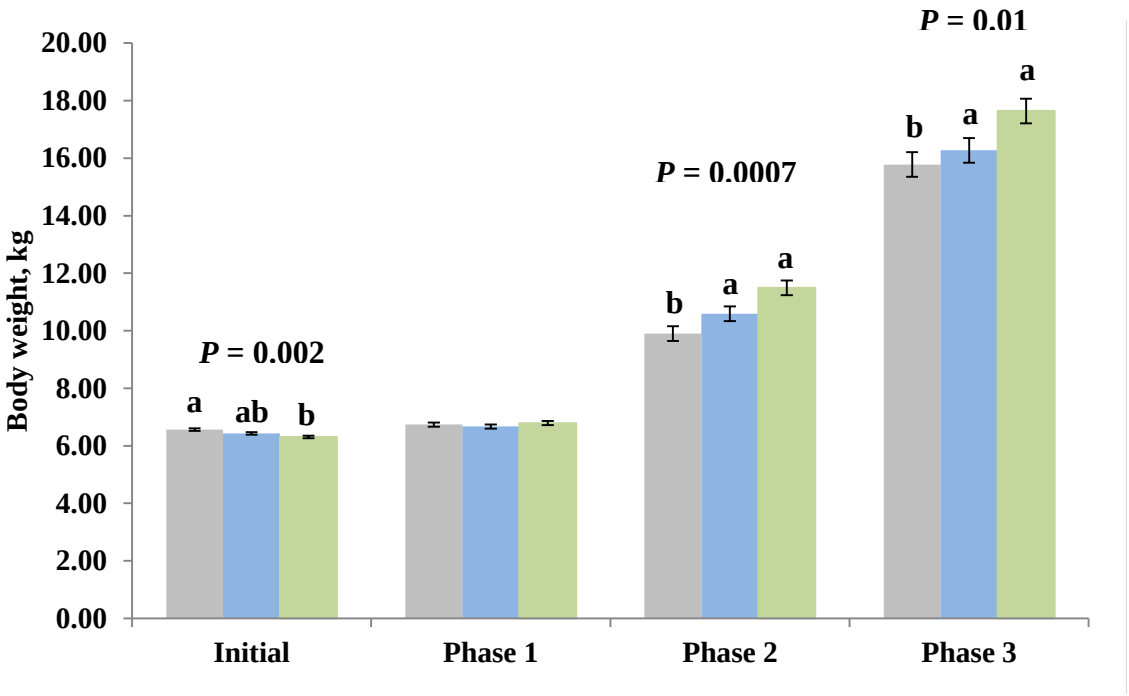

Figure 11. Main Effect of *Pichia guilliermondii* (Pg) fed in the sow diet on body weight of nursery pigs (Exp. 2). SC – Sow gestation/lactation control; S1 – Sow gestation/lactation control + 0.1% Pg; S2 – Sow gestation/lactation control + 0.2% Pg. Bars with different letters differ ($P < 0.05$).

CONCLUSION

Supplementation of a control diet with an additional 1% arginine from d 93 to 110 of gestation increased body weight gain in gilts and parity 1 sows. However, this increase in weight was independent of litter weight and had no effect on litter size at farrowing or subsequent lactation performance as determined by litter weaning weight and litter weight gain. Further research is warranted to determine the optimal time and level of arginine supplementation during gestation to achieve the greatest impact on litter and reproductive performance.

Supplementation of standard gestation and lactation diets with *Pichia guilliermondii* may be beneficial to sow reproductive performance, as well as the performance of the offspring as manifested in an improvement in litter size at birth, a reduction in light weight pigs at birth, and an increase in litter weaning weight. Additionally, it appears that supplementation of *Pichia guilliermondii* to gestation and lactation diets alters the immune profile of sow serum, but not colostrum or milk, when provided throughout gestation and lactation. Continued inclusion of the whole yeast product in nursery diets improved weaned pig performance, and may be additive. Inclusion of *Pichia guilliermondii* in nursery diets improved average daily gain as well as feed intake as the level of *Pichia guilliermondii* increased in sow diets. Finally, *Pichia guilliermondii* inclusion in sow diets was also beneficial to gain:feed in pigs receiving control nursery diets. It appears inclusion of *Pichia guilliermondii* during gestation and lactation, especially in pigs from sows provided diets containing 0.1% *Pichia guilliermondii*, impacted immune response to LPS as pigs from sows provided diets containing 0.1% *Pichia guilliermondii* had a decrease in the concentration of neutrophils in the blood, as well as a decrease in the neutrophil:lymphocyte ratio, and a swift, but robust, increase in the inflammatory cytokines IL-1β and IL-6 at 5 h post-challenge. Thus, *Pichia guilliermondii* may be a viable alternative to other yeast culture

products in its ability to improve reproductive and growth performance, perhaps acting as an immunomodulator.