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INFLUENCES OF SUPPLEMENTING A MANNAN OLIGOSACCHARIDE CONTAINING PRODUCT TO PIG DIETS ON SOW AND WEANLING PIG PERFORMANCE

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ABSTRACT OF THESIS

INFLUENCES OF SUPPLEMENTING A MANNAN OLIGOSACCHARIDE CONTAINING PRODUCT TO PIG DIETS ON SOW AND WEANLING PIG PERFORMANCE

The objective of these studies was to evaluate the effect of supplying mannan oligosaccharides (MOS) to pig diets on both weanling pig and sow performances.

Nursery pigs challenged with LPS to stimulate an immune challenge had increased body temperature and respiratory rate as well as elevated serum cortisol and TNF-α concentration, MOS-supplemented pigs had a lower rectal temperature and respiratory rate which implied that MOS improves some aspect of the immune function of piglets.

MOS supplementation in sow diets during late gestation and lactation had no effect on litter size, but the piglets from MOS-fed sows were heavier at birth (P = 0.04), at weaning (P = 0.03), and during the entire nursery period (P < 0.01). Moreover, milk fat and protein levels as well as the Ig concentrations in milk from MOS-fed sows were numerically higher (2 – 12%; P > 0.10) than control sows.

Overall, MOS supplementation in the nursery diet may have limited effects on the growth performance, but may have some beneficial influence on pigs under immune challenge. Furthermore, including MOS to the sow diet during late gestation and lactation can potentially improve piglet body weight as well as growth during the suckling and nursery periods.

KEYWORDS: Mannan oligosaccharide, Weaned pig, Immunity, Sow, Reproduction.

I-Fen Hung

December 2nd, 2009
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INFLUENCES OF SUPPLEMENTING A MANNAN OLIGOSACCHARIDE CONTAINING PRODUCT TO PIG DIETS ON SOW AND WEANLING PIG PERFORMANCE

THESIS

An abstract of a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture at the University of Kentucky

By
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Lexington, KY

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Lexington, KY
2009

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Chapter 1. Introduction

Mannan oligosaccharides (MOS) are complex sugars which consist mainly of mannose that are found mostly in the cell walls of yeast and plants. MOS was first introduced as a feed additive in 1993 (Hooge, 2003). It has been suggested that dietary MOS has benefits in improving gut health by reducing pathogen colonization (Newman, 1994; Castillo et al., 2008).

Most enterobacteria have a specific organelle, type 1 fimbria, which mediates binding to mannose on the intestinal wall (Firon et al., 1983). After they adhere, the pathogens colonize and proliferate in the intestine, which consequently causes disease. When dietary MOS is presented, pathogens which normally adhere to mannans on the mucosal surface of the intestine may instead bind to the mannan component of products. After this, pathogens are flushed from the intestinal tract rather than colonize in the intestine (Newman, 1994).

There are many studies showing that adding MOS to the diet has improved the growth performance of broiler chickens (reviewed by Hooge, 2004a), turkeys (reviewed by Hooge, 2004b), calves (reviewed by Hooge, 2006) and pigs (reviewed by Pettigrew, 2000). There are also other studies demonstrating that dietary MOS aids the immune system and gut health of broiler chickens (Spring et al., 2000), turkeys (Savage et al., 1996), calves (Franklin et al. 2005), and rabbits (Mourão et al., 2006).

In swine, the immune system is not fully developed at birth, and thus it is important to improve the neonatal piglets’ immunity through sow’s milk and the cleanliness of the environment. Studies have shown that adding MOS to sow’s diet increases piglet weight at birth and weaning, improves pre-weaning growth, and raises survival rate (Pettigrew et al. 2005). Also, Spring and Geliot (2003) observed
that, adding MOS to the gestation diet 8 weeks prior to farrowing reduced the number of coliforms and Clostridium perfringens in the feces of the sow during farrowing.

Therefore, the objective of the current research was to evaluate the effect of supplying mannan oligosaccharide to nursery pig on growth performance and immune challenge (Chapter 3) and to gestating and lactating sow on reproductive performance and milk quality in conjunction with continuing pigs born to these sows on a MOS study in the nursery (Chapter 4).
Chapter 2. Literature review

2.1 Health and nutrition

2.1.1 Sow nutrition

The productive efficiency of a sow is the most important biological key to economic profitability of a pig farm apart from a disease outbreak. Sows with low productive efficiency are characterized by smaller litters, weaker piglets, or longer period of postweaning anestrus. Although the productive efficiency is influenced by improved genotype, management, and facilities, nutrition is a primary factor to obtain the maximum potential of the sow. Feeding strategy during pregnancy is important because it determines the size and subsequent viability of piglets at birth, the amount of mammary tissue the sow contains at parturition, and as well the potential milk production of the sow during lactation (Pluske et al., 1995).

There are many nutrients that affect sows’ performance, including energy, protein, minerals, and vitamins. Energy and protein are the factors that are directly used for growth and maintenance. This literature review mainly focuses, therefore, on the energy and protein/amino acids requirements for the sows.

Energy for sow diets is used for maintaining normal body functions, growth of fetal, placental, uterine, and mammary tissues, and deposition in maternal body tissues. Maintenance of the sow and the growth of the embryo are considered to receive first priority for the nutrients. Once those priority needs are satisfied, the extra nutrients are deposited in maternal tissues (Trottier and Johnston, 2001). However, if there are any insufficient dietary nutrients for the priority needs, those nutrients will be withdrawn from the sow’s body tissue, and thus may subsequently cause reproductive failure. Johnston et al. (1989) and Trottier and Johnston (2001) stated that reduced weight gain during gestation due to insufficient energy intake may still
allow successful lactation, but results in thinner sows at weaning and delayed postweaning estrus.

The feed and energy requirements of the pregnant sow will vary with her body weight, target body weight gain during pregnancy, and other management and environmental parameters. NRC (1998) suggested the daily maintenance energy requirement as 106 kcal ME/kg metabolic body size (BW$^{0.75}$). At the early period of gestation, the nutrients in the diet should be sufficient not only for the maintenance and growth of the sow, but also for the conception, and for the developing embryo/fetus. NRC (1998) also recommends that the daily energy requirement for protein accretion is 10.6 kcal of ME/g; for fat accretion is 12.5 kcal of ME/g; for the growth of the fetus is 35.8 kcal of ME per pig in the litter.

This program for calculating the energy requirement for gestating sows is easy to apply. However, this single-phase feeding strategy may not be appropriate to support the nutrient needs of pregnant sows since their weight gain and fetus growth changes during gestation (Ji et al., 2005; McPherson et al., 2004), being an accelerated rate in late gestation. Ji et al. (2005) suggested that an optimal feeding strategy, i.e., 2-phase feeding, for pregnant sows should be sufficiently flexible to adjust for the nutrient allowance of gilts according to their nutrient needs for both maternal and fetal growth.

Although increased sow feed intake during gestation increases the growth of the fetus and deposition of body fat and protein, it may cause reduced energy intake and increased weight loss during lactation (Cooper et al., 2001). Therefore, it is desirable to limit energy intake during pregnancy to control weight gain (NRC, 1998). However, fetal growth increases dramatically in late gestation with 60% of growth occurring during the last 30 days of gestation (Pluske et al., 1995), which
demonstrates that it is necessary to increase feed intake during this period to accommodate the increased nutrients required for rapid fetus growth.

During late gestation (d 50 to 110), the ME requirement for sows is increased from 3 to 12% of total maternal energy intake. This period is also the occurrence of the acceleration in fetal weight (Pluske et al., 1995). In addition, milk production is a gradually accumulated process in mammary glands during gestation. Since the colostrum and milk are the main sources of energy and passive immunity for the neonatal pigs, the quantity and the quality of colostrum and milk are extremely important for having healthy piglets.

After parturition, sow feed intake is even more important than it is during gestation. This is because the feed intake directly affects the growth and development of the neonatal piglets via the milk produced and influences the reproductive efficiency of the sow in the next parity. Sows with sufficient nutrient intake influence the nutrient composition in the milk that subsequently affects the piglets’ growth and health (Trottier and Johnston, 2001). On the other hand, insufficient nutrient intake will compromise the sow’s body composition, hence it takes time for the sow to recover and return to service. Koketsu et al. (1996a) has shown that sows with either low feed intake throughout lactation or low feed intake at various times during lactation are more likely to have lower litter size at weaning and a longer period before the next estrus.

The daily energy needs during lactation include a requirement for maintenance of the sow and for milk production. The energy requirement for milk production can be estimated from the growth rate of the suckling pig and the number of pigs in the litter. The equation of estimated milk gross energy (GE) is derived from Noblet and Etienne (1989) where GE per day = (4.92 × ADG × number of pigs) - (90 × number
of pigs). This amount is then converted to dietary energy where dietary ME = (6.83 × ADG × number of pigs) - (125 × number of pigs) (NRC, 1998).

Among the essential amino acids, lysine is considered to be the first limiting amino acid for establishing the ideal protein for both gestation and lactation. The daily requirement for lysine is the sum of the requirements for maintenance and for milk production, with a reduction to account for the use of the sow’s body protein to provide part of the lysine needed for milk production. The daily maintenance requirement for true ileal digestible lysine during gestation and lactation is considered to be 36 mg/kg BW^{0.75}; the requirement for milk production is suggested to be 22 g of apparent ileal digestible lysine/kg of litter weight gain (NRC, 1998).

Many studies found that the survival rate of a heavier piglet at birth is higher than that of a lighter piglet (Smith et al., 2007; Tokach et al., 1992; Hall et al., 1987). The heavier piglet at birth is also heavier at weaning (Smith et al., 2007; Tokach et al., 1992). Furthermore, it has been studied that the weaning weight of a pig affects its growth rate during the growth-finishing period and its carcass characteristic (Gondret et al., 2005; Gondret et al., 2006; Wolter and Ellis, 2001). Similarly, a young, fast growing pig will also tend to be a healthy piglet because its immune system is promoted by rapid growth (Gadd, 2003). The good immune status reduces the possibility of being attacked by the pathogens and subsequently helps this fast growing piglet to be a fast growing nursery, and then finisher pig.

Having heavier and healthier piglets costs the producer less money to feed and house as the pigs reach market weight earlier than lighter pigs. It also increases the profit from larger litter size and lower mortality of the piglets. Therefore, the nutrition for pregnant sows becomes an important issue for the swine nutritionist.
2.1.2 Neonatal piglet nutrition

Major body constituents which potentially are used as energy-yielding substrates for new born piglets are protein, glycogen, and fats (Le Dividich et al., 2005). At birth, neonatal piglets have really low energy reserves in the body, including 1-2% fat, 8-25% liver glycogen, and approximately 12% protein (Pluske et al., 1995; Le Dividich et al., 1991; Elliot and Lodge, 1977). However, due to the dramatically and rapidly depleted glycogen reserve within 12 hours post-partum (Elliot and Lodge, 1997), a varying low rate of protein catabolism and a low amount of body fat content, colostrum becomes the major energy source for neonatal piglets.

Many digestive enzymes are present at birth (Figure 2.1). The major energy source for neonates in colostrum is fat and lactose. According to the research of Le Dividich et al. (1994), 59% of fat and 100% of lactose are digested. Lactose concentration in the colostrum is 3.4%, while its concentration in milk is 5.3% (Darragh and Moughan, 1998; Klobasa et al., 1987; Trottier and Johnston, 2001). Hydrolyzed lactose yields glucose and galactose which are readily absorbed into the piglet’s blood and are either metabolized directly, or used to be stored in the liver and muscle as glycogen (Darragh and Monghan, 1998). Veum and Odle (2001) summarized that lactase activities are high at birth, reaching a maximum at 1 week before declining to 6 or 8 weeks of age; maltase activity is low in intestinal mucosa at birth, but increases gradually from birth to 6 or 8 weeks of age; also sucrase activity is not found at birth, but is present by 1 week of age.
The fat content in sow colostrum and milk are 5.9% and 7.9%, respectively (Darragh and Moughan, 1998; Klobasa et al., 1987; Trottier and Johnston, 2001). Pancreatic lipase activities are high at birth, drop transiently with the onset of suckling and weaning, and then increase with age (Lindemann et al., 1986; Veum and Odle, 2001). The high level of lipase and lactase helps neonates digest fat and lactose in colostrum faster, thereby helping them to utilize the energy for growth, activity, and heat production.

The quantity and quality of colostrum is influenced by sow genotype, parity, endocrine status, nutrition, environment, litter characteristics, or a combination of these factors (Farmer and Quesnel, 2009). On average, the content of fat, protein, and lactose in colostrum is 5.9%, 15.1%, and 3.4%, respectively; the content of fat, protein, and lactose in milk is 7.6%, 5.5%, and 5.3%, respectively (Darragh and Moughan, 1998; Klobasa et al., 1987; Trottier and Johnston, 2001).
Table 2.1 Protein content in colostrum and in milk (adapted from Darragh and Moughan, 1998).

<table>
<thead>
<tr>
<th></th>
<th>Colostrum&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Milk&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein, g/100g milk</td>
<td>15.14</td>
<td>5.47</td>
</tr>
<tr>
<td>Casein, g/100g milk</td>
<td>1.48</td>
<td>2.74</td>
</tr>
<tr>
<td>Whey Protein, g/100g milk</td>
<td>14.75</td>
<td>2.22</td>
</tr>
<tr>
<td>IgG, mg/mL milk&lt;sup&gt;3&lt;/sup&gt;</td>
<td>95.6</td>
<td>0.9</td>
</tr>
<tr>
<td>IgA, mg/mL milk&lt;sup&gt;3&lt;/sup&gt;</td>
<td>21.2</td>
<td>5.3</td>
</tr>
<tr>
<td>IgM, mg/mL milk&lt;sup&gt;3&lt;/sup&gt;</td>
<td>9.1</td>
<td>1.4</td>
</tr>
</tbody>
</table>

<sup>1</sup> Taken immediately postpartum  
<sup>2</sup> Classified as milk samples collected between 14 and 21 days postpartum.  
<sup>3</sup> The total Ig in colostrum is 125 mg/mL which is equivalent to 12.5 g/100mL

Proteins are the predominant components of total solids in the colostrum (Table 2.1). There are 2 groups of proteins in colostrum and milk, including caseins and whey proteins. Casein is the main source of dietary essential amino acids; it also works as the carrier of calcium which helps the absorption of calcium. The study from Le Dividich et al. (1994) showed that colostral energy and nitrogen are very efficiently utilized by the neonates, where the efficiency of ME utilization for protein and fat synthesis and of glycogen degradation is approximately 0.91. Conversely, whey proteins include blood serum albumin, α-lactoalbumin, β-lactoglobulin, Immunoglobulin G (IgG), Immunoglobulin A (IgA), Immunoglobulin M (IgM), lactoferrin, and other minor proteins.

Colostrum has a high concentration of total solids and protein but only low levels of fat and lactose. At the same time, the low proteolytic activity in the gastrointestinal tract of the neonatal piglets as well as the protease inhibitor in the colostrum allows the newborn piglet to ingest colostral contents (Pluske et al., 1995; Le Dividich et al., 2005). The majority of proteins in colostrum are whey proteins (14.75% of total colostrum; Darragh and Moughan, 1998), but the percentage of
protein dramatically decreases within 24 hours post partum (Klobasa et al., 1987). In milk, the proportion of casein and whey proteins to milk are similar (2.74 % and 2.22 %, respectively; Darragh and Moughan, 1998).

Placental transfer of immune antibodies is almost nil in the pig (Pond, 1973). Although most components of the immune system of the piglet are present at birth, it takes several weeks of life to become functionally developed. Thus, maternal immunoglobulin needs to be provided for passive immune protection until the piglets are able to synthesize adequate amounts of antibodies (Le Dividich et al., 2005). Due to the low activities of the gastric and pancreatic proteolytic enzymes in neonatal pigs at birth and protease inhibitors in colostrum, piglets can absorb intact immunoglobulin directly into their blood stream (Le Dividich et al., 2005).

IgG is the predominant immunoglobulin in the colostrum (76% of total immunoglobulin), but its concentration in milk rapidly declines during the first 24 hours after parturition (approximately 85% of reduction; Klobasa et al., 1987). The concentration of IgA and IgM also declines with time, but the reduction is not as pronounced as in IgG. Thus, IgA becomes the dominant immunoglobulin in mature milk. Darragh and Moughan (1998) stated that the shift of the immunoglobulin level reflects the changing needs of the piglet, as absorption of whole protein gives way to the maintenance of localized immune protection within the gut.

In addition to the immunoglobulin, there are some other cells in colostrum and milk that help provide protection against infection in mammary glands and piglets’ gastrointestinal tract, including neutrophils, lymphocytes, macrophages, and epithelial cells (Darragh and Moughan, 1998). Leukocytes in colostrum are absorbed intracellular in the upper small intestine, enter the lymphatic vessels, and are
transported to the mesenteric lymph nodes, which then stimulate the development of cellular immunity of the neonates (Blecha, 1998).

The energy and immunoglobulin in colostrum not only helps piglet growth and defense against disease, but also aids the development of the gastrointestinal tract and regulation of blood glucose of piglets (Devillers et al., 2004). Colostrum and milk contain high levels of growth factors that accelerate proliferation and maturation of the gut in neonatal animals (Kelly and King, 2001).

2.1.3 Weaning piglet nutrition

Piglets are usually weaned between 16 and 18 weeks of age in the semi-natural environment (Jensen and Recén, 1989). However, in a practical farm system, piglets are weaned between the age of 2 to 6 weeks which is prior to their digestive and immune systems being fully developed (Bailey et al., 2005). Regardless of the age of weaning, the various stressors, including physical stress (i.e. separation from dam, movement to new environment, regrouping with new pen mates, and building new social orders) and nutritional stress (i.e. feed type changing from liquid sow milk to solid feed), cause the reduction of growth performance and immunocompetence during this period (Blecha and Kelley, 1981).

A high weaning weight usually implies rapid and healthy growth after weaning and all the way through to slaughter (Wolter and Ellis, 2001). The post-weaning challenges cause the deterioration of health and growth which increases costs in the industry, including a longer feeding period before reaching market weight, less turnover rate of the facility, and possible requirements for specific nutrient supplements for weaker pigs. Therefore, the most important mission for the
nutritionist to deal with the post-weaning growth check is to reduce the stress during weaning transition and, thereby, to improve growth and immunity.

The nutrients used as energy sources in animal feed are carbohydrates and fats. According to NRC (1998), the energy level in the diet for pigs weighing 3 to 20 kg should exceed 3265 kcal/kg of metabolizable energy (ME). McNutt and Ewan (1984) estimate that the ME requirement for maintenance for the 4-week-old weaned pig is 115 kcal/day/kg^{0.75}. However, feed intake is usually low in the first days after weaning due to the unfamiliarity of the pig to the feed type and other stresses. This indicates that the energy intake is not sufficient. A high energy-content-diet may increase energy intake only if the right energy source is chosen. Fat utilization is inefficient at weaning because of the low lipase activity during this period (Lindemann et al., 1986; Maxwell and Carter, 2001). The energy sources for optimal utilization in newly weaned pigs are lactose, glucose, or sucrose, in dried whey or dried skim milk (Maxwell and Carter, 2001; Owsley et al., 1986).

Proteins are complex components that form from various combinations of amino acids and/or other compounds, i.e. carbohydrates or lipids. Amino acids are the basic structure for body tissues, including muscles, skin, connective tissue, nerves, and organs. Amino acids are classified by their essentiality to the dietary protein requirement. The non-essential amino acids are those that can be synthesized by the animal itself while the essential amino acids have to be provided in the diet. To attain optimal growth, the dietary amino acid content must be balanced.

Lysine has been considered the limiting amino acid in the porcine diet. Many studies indicate that pigs which are fed higher lysine levels than that recommended by NRC (1998) have a higher growth rate and improved feed efficiency (Maxwell and Carter, 2001). Under the concept of ideal protein, suggested by Fuller and Wang
(1990), Chung and Baker (1992), and Cole and Van Lunen (1994), the amino acid requirements are presented as a ratio to lysine.

Vitamins and minerals are also important for growth of tissue and maintaining or aiding the regulation of the body. Ca and P are the major components of the skeleton while vitamin D is involved in the regulation of Ca and P absorption and accumulation in bones. Ca is quite low in cereal grains and most plant protein ingredients, thus most Ca is supplied by inorganic sources. In contrast to Ca, most cereal grains and most plant protein ingredients contain sufficient levels of P, but it is stored as a phytic phosphate which is almost indigestible by pigs. Moreover, phytate has the potential to form insoluble salts with Ca, Fe, Zn, Mn, and Cu (Shelton et al., 2004), which may decrease the availability of these minerals. Therefore, the needs of P and these minerals have to be added in an inorganic source in the same way as Ca. The alternative method is adding phytase into the diet. Phytase hydrolize the undigestible phytate and thus releases the phytic P, which increases the phosphorus bioavailability (Yi et al., 1996).

Most vitamins and minerals regulate the bodily functions as cofactors of enzymes as well as directly influencing the tissue. For instance, Cu is a component of amine oxidase that inactivates and catabolizes active biogenic amines (Linder, 1991). Enzymes that contain Zn protect the membrane integrity against peroxidative damage (Hill and Spears, 2001). Coffey et al. (1994) and Hill et al. (2000) showed that high dietary levels of Cu and/or Zn improved growth performance of weaning pigs which may be through effects on the microbial population of the gastrointestinal tract.
2.1.4 Gut health

During suckling, there are some growth factors in milk, such as epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1), which contribute to neonatal gastrointestinal development (Blecha, 1998; Odle et al., 1996). It has been found that EGF and IGF-1 stimulate the activity of the digestive enzymes in the neonatal piglet’s intestine and increase villus length (reviewed by Odle et al., 1996). Kingsnorth et al. (1990; cited by Odle et al., 1996) suggest that supply EGF to 20-kg pig diet may aid in the recovery of traumatized gastric and intestinal tissues.

Nevertheless, the removal of these growth factors from milk and the stress during weaning causes the change of the intestine morphology. Villous atrophy and crypt hyperplasia were believed to be a result of stress, but there is evidence that it is more closely related to the reduction of feed intake over the weaning transition (Kelly and King, 2001, Spreeuwenberg et al., 2001). Yet the low feed intake is also an effect caused by stress. It was observed that some newly weaned pigs may take 3 minutes to consume their first meal, but still some others may take even longer (up to 56 hours) to discover the food (Brooks et al., 2001). The morphological change as well as the decreased digestive enzyme activity influence the digestive and absorptive capacity and may lead to malabsorption, dehydration, enteric infection, and diarrhea.

Improving feed intake during the immediate post-weaning period is very important for the development of the small intestine and subsequent growth performance (Pluske et al., 1995; McCracken et al., 1999). There are marked changes to the structure and function of the small intestine that happen within 24 hours of weaning, and generally comprise a decrease in villous height and an increase in crypt depth as well as reductions in the specific activity of the digestive enzymes, such as lactase and sucrase and a reduced absorptive capacity (Pluske et al., 1995). Epithelial
cells need energy to maintain gut integrity. Glutamine is considered as the fuel for enterocytes and provides amine group to support their metabolism as well as the structure and the function of the small intestine (Pluske et al., 1997). The effect of post-weaning low feed intake on small-intestinal architecture might be ameliorated by providing adequate energy for the epithelial cells (Vente-Spreeuwenberg et al., 2003).

In addition to stress and low feed intake, the enterobacteria population may also contribute to the growth reduction and disease during weaning. The intestine of a newborn animal is considered germ-free (Maxwell and Stewart, 1995). However, soon after the neonates are born and consume their first meal, the intestine will be colonized by the gut bacteria which are from the birth canal, teat, sow feces, and the environment. These pathogens are controlled by maternal IgA (Kelly and King, 2001). However, after weaning, these pathogens use the undigested chyme and sloughed intestinal cells as their energy source to colonize and proliferate (Pluske et al., 1997).

Kelly (2004) stated that the most important step to establish enterobacterial infection is the attachment for pathogen to host tissue. The attached bacteria regulate the gene program in intestinal epithelial cells and thus influence the expression of epithelial cell products that subsequently alter the physiological and biochemical functions of intestinal barriers (Kelly, 2004). There are many studies interested in improving weaning pig’s intestinal morphology, digestive enzyme activity, and intestinal bacteria population. Most studies concentrate on probiotics (e.g., lactobacillus, bifidobacteria, yeast, etc.) and/or prebiotics (organic acids, oligosaccharides, etc.) in the piglet’s diet.

Lactic acid bacteria (LAB), such as lactobacilli and bifidobacteria, compete within the binding sites in the intestine and thus reduce the colonization of the pathogens, e.g., *E. Coli* and *Salmonella*. In addition, LAB produces lactate which
reduces the intestinal pH and consequently suppresses the growth of the pathogens. Pollmann et al. (1980) showed that adding a microbial feed additive (lactic acid-producing bacteria) with an antibiotic (virginiamycin) to a weaning pig’s diet improves the average daily gain and feed/gain ratio. Similarly, Huang et al. (2004) indicated that lactobacilli supplementation in the drinking water of weaning pigs decreased E. coli and aerobe counts (P < 0.01) but increased Lactobacilli and anaerobe counts (P < 0.01) in digesta and mucosa of most sections of the GI tract.

Organic acids reduce gastric pH, which subsequently influences the activities of the digestive enzymes. Because of this acidic environment, the intestinal microflora activities are suppressed and thus reduce the incidence of diarrhea. It has been observed that supplying organic acid, such as citric, fumaric, and lactic acid, significantly improved the growth performance and feed efficiency of weaning pigs (Falkowski and Aherne, 1984; Burnell et al., 1988; Radcliffe et al., 1998). Furthermore, Tsiloyiannis et al. (2001) indicated that organic acid supplementation reduce incidents of diarrhea, thus increasing grow performance.

Yeast contains enzymes, vitamins, and other nutrients or growth factors that have been said to produce beneficial production responses in pigs (Kornegay et al., 1995). Yeast also changes the microflora population in the gut, due to its cell wall components (mannans) or directly due to the effect of the competition for the binding site on the intestinal wall with the pathogens, which reduces pathogens and toxic metabolites and subsequently improves animal health and growth performance (van Heugten et al., 2003a). Mathew et al. (1998) concluded that the addition of yeast culture to the diets improved feed intake and may have potential benefits of reducing the E. Coli count in the gastrointestinal tract. However, the results among experiments are not consistent.
The optimal nutrition is important to have healthy pigs which consequently increase the profit of the pig producer. Healthy pigs need fewer days to reach market weight and have the potential to yield more lean muscle. However, balanced nutrient supply is not enough. Improving immunocompetence of the pigs provides additive effects for pig’s health.

2.2 Immunocompetence

2.2.1 Immune system

The immune system is a natural defense of a creature that self-protects the body. There are 2 types of immunity, innate immunity and adaptive immunity. Innate immunity is the first line of defense against common microorganisms. It is also called non-specific immunity that involves phagocytes (macrophages, neutrophil, and granulocytes), natural killer cells, and the complement system. Once pathogens perforate the epithelial barriers, macrophages and other leukocytes recognize the pathogens through the receptors on the surface which triggers the engulfment of the bacteria and stimulate the release of cytokines and chemokines. Cytokines affect the behavior of other cells which have the receptors for cytokines, whereas chemokine attract and activate cells with specific chemokine receptors such as macrophages and neutrophil (Janeway et al., 2005).

In contrast, adaptive immunity, which is activated by the innate immunity, consists of lymphocytes which are stimulated by foreign microorganisms’ antigens or their product, thus releasing antibodies to protect the body. Adaptive immunity provides the ability to recognize and remember the specific pathogen thus responding faster when exposed to that pathogen again. Adaptive immune response comprises 2
types: humoral and cell-mediated immunity. Humoral immunity is mediated by the antibodies that are produced by B-lymphocytes (B-cells) while cell-mediated immunity is modulated by T-lymphocytes (T-cells) and their released cytokines as well as macrophage and natural-killer cells. The antigen-recognition molecules of B-cells are the immunoglobulins; each B-cell produces its specific immunoglobulins. In contrast, T-cells do not recognize and bind antigen directly. The specific membrane glycoprotein, called major histocompatibility complex (MHC), display peptide antigen to T-cells, thus activates T-cells (Janeway et al., 2005).

T cells are divided into 2 subsets, including cytotoxic T cell (T_{C} cell) and helper T cell (T_{H} cell). Cytotoxic T cell expresses the glycoprotein called CD8 which recognizes the class I MHC molecules of the antigen and thus releases cytotoxins to kill the infected cell. In contrast, the T_{H} cell expresses CD4 that recognizes class II MHC molecules. T_{H} cells include 2 subtypes; Type 1 T_{H} cell activates macrophage activity while type 2 T_{H} cell stimulates B cell to produce antibodies. The functions of each leukocyte are listed in Table 2.2.
Table 2.2 Functions of leukocytes that are involved in immunity (Johnson et al., 2001).

<table>
<thead>
<tr>
<th>Leukocyte</th>
<th>Primary function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Innate immunity</strong></td>
</tr>
<tr>
<td>Neutrophil</td>
<td>Phagocytosis and destruction of bacteria</td>
</tr>
<tr>
<td></td>
<td>Produce inflammatory response mediators</td>
</tr>
<tr>
<td>Macrophage</td>
<td>Phagocytosis and destruction of bacteria</td>
</tr>
<tr>
<td></td>
<td>Produce inflammatory cytokines that activate other leukocytes and that initiate</td>
</tr>
<tr>
<td></td>
<td>other components of the acute-phase response</td>
</tr>
<tr>
<td></td>
<td>Antigen presentation</td>
</tr>
<tr>
<td>Natural killer cell</td>
<td>Provide early defense against viruses and certain intracellular pathogens</td>
</tr>
<tr>
<td></td>
<td><strong>Adaptive immunity</strong></td>
</tr>
<tr>
<td>B lymphocyte</td>
<td>Produce antibody</td>
</tr>
<tr>
<td></td>
<td>Antigen presentation</td>
</tr>
<tr>
<td>T lymphocyte</td>
<td>Combat intracellular pathogens by activating macrophages (inflammatory CD4 T</td>
</tr>
<tr>
<td></td>
<td>cells, T_{H1})</td>
</tr>
<tr>
<td></td>
<td>Combat extracellular pathogens by stimulating B cells to produce antibody (helper</td>
</tr>
<tr>
<td></td>
<td>CD4 T cell, T_{H2})</td>
</tr>
<tr>
<td></td>
<td>Destroy infected cells (CD8 cytotoxic T cells)</td>
</tr>
</tbody>
</table>

Clonal selection and clonal expansion are the basic principle of adaptive immunity. The receptor of each lymphocyte recognizes its specific antigen when it encounters foreign bacteria and then activates that lymphocyte. These activated cells then proliferate and differentiate into the antigen-specific effector cells. After the antigen has been eliminated, the response ceases, but the memory cells sustain the immunity. Once the pathogen is encountered the second time, the memory cells can rapidly proliferate and differentiate into the effector cells to discard the pathogen. The responses of the immune system are illustrates in Table 2.3.
Cytokines are proteins made by cells that allow cellular communication between the innate and adaptive immune responses as well as between the immune system and other cells. There are 2 major structural groups of cytokines: the hematopoietin family and the tumor necrosis factor (TNF) family. The hematopoietin family comprises growth hormones and interleukins that influence both innate and adaptive immunity. Additionally, cytokines from the TNF family also function in both innate and adaptive immunity and include many members that are membrane-bound. Table 2.4 shows the common cytokines in the body and their functions.
Table 2.4. Important cytokines response to bacterial products (Janeway et al., 2005).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Functions</th>
<th>System effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>Activate vascular endothelium</td>
<td>Fever</td>
</tr>
<tr>
<td></td>
<td>Activate lymphocytes</td>
<td>Production of IL-6</td>
</tr>
<tr>
<td></td>
<td>Local tissue destruction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increases access of effector cells</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Lymphocyte activation</td>
<td>Fever</td>
</tr>
<tr>
<td></td>
<td>Increased antibody production</td>
<td>Induces acute-phase protein production</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Activate vascular endothelium and increases vascular permeability, which leads to increased entry of IgG, complement, and cells to tissue and increased fluid drainage to lymph nodes</td>
<td>Fever</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mobilization of metabolites</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shock</td>
</tr>
</tbody>
</table>

2.2.2 Nutrients and immunity

Nutrition requirements established by NRC are considered as the minimum requirements for maintaining normal growth, health and productivity. NRC (1998) also suggested that the “safety-margin” should be added to the suggested amount. Meanwhile, the relationship between nutrition and immunity can be complicated. Malnutrition or dystrophy is a possible reason for the reduced immune competence of weanling pigs and consequently increases the susceptibility to diseases. Either an excess or deficiency of energy and/or protein as well as unbalanced vitamins and minerals are factors that may cause this result.

Energy may not directly affect the immune system, but it influences feed intake and that determines the intake of other nutrients, such as amino acids, vitamins, and minerals. With regard to protein, restricted protein level in the sow diet may
reduce the piglet’s survival rate and growth rate as well as the milk yield (DeGeeter et al., 1972). However, the protein level (9% vs. 18%) in the sow diet during gestation will not influence the antibody synthesis of the piglet and the concentration of IgG, IgA, or IgM in the piglet’s serum (Haye et al., 1981). This result suggests that optimal restricting of the protein intake of the sow will not affect piglet synthesizing antibodies or absorbing immunoglobulin (Kelley and Easter, 1991). NRC (1998) suggested that the CP requirement in the gestation sow’s diet is 12 – 13% based on corn-soybean meal diet, depending on the sow’s weight at breeding and/or the expected litter size.

Amino acids are important in the proper functioning of the immune system. For instance, arginine stimulates not only the secretion of growth hormone and insulin that beneficially modulate the immune response, but also the production of nitric oxide (NO) that aids macrophages and neutrophils to kill the engulfed microorganism (Wu et al., 2004, Kim and Wu, 2009). Furthermore, Johnson et al. (2001) stated that glutamine is essential for the normal functioning of macrophages and lymphocytes during an immune response. Additionally, Wu et al. (1996) demonstrated that dietary glutamine supplementation prevented intestinal atrophy during week 1 post-weaning and thus improved the pig’s growth performance consequently.

Vitamins and minerals function with, or in conjunction with, many enzymes and cells that regulate the normal functions of body tissues and aid the immune system. Vitamin A, C and E are so called antioxidant vitamins that help protect the cell integrity, and thus are beneficial to immunity. Vitamin E has antioxidant properties that protect cells from peroxidation. Meanwhile, Se is an integral component of glutathione peroxidase and is a complementary role to vitamin E. Peplowski et al. (1981) suggested that vitamin E and Se deficiency may retard the
production of humoral antibodies. Also, supply of vitamin E and Se in excess of the
requirement level increase antibody production and lymphocyte proliferation in pigs
(Larsen and Tollersrud, 1981; Toepfer-Berget al., 2004).

Vitamin A deficiency influences the integrity of mucosal epithelial cells in the
respiratory, gastrointestinal, and uterine tracts (Johnson et al., 2001). In addition,
Vitamin A deficiency decreases lymphocyte proliferation, antigen-specific antibody
production and T-lymphocyte proliferation in vitro, and increases bacterial adherence
to respiratory epithelial cells (Chandra, 1993; Friedman and Sklan, 1989). Harmon et
al. (1963) observed that deficient dietary vitamin A decreases the albumin level in the
serum and increases α-globulin level and γ-globulin level in weaning pigs. Elevated
globulin level in the serum may indicate the chronic clinic responses, such as
pathogen infection, liver disease, or kidney dysfunction (Coons et al., 1955).

Zn deficiency damages epidermal cells, resulting in skin lesions, which
represent the loss of the first line of defense of immunity. Zn deficiency also affects
other mediators of nonspecific immunity, such as polymorphonuclear leukocyte
function, natural killer cell function, and complement activity. An excess 80 ppm of
dietary Zn over the requirement improved macrophage function after stimulation by
phytohemagglutinin (PHA) or pokeweed mitogen (PWM), which suggested that the
Zn requirement for the immune system in swine is greater than that necessary for
growth performance (van Heugten et al., 2003b).

Iron (Fe) is an essential component of hemoglobin which carries oxygen in the
blood, but it is also an important nutrient for pathogens. Knight et al. (1983) observed
that excess dose of Fe may stimulate the growth of pathogens and thus affect the pigs’
health. Removing Fe from blood to make it inaccessible to pathogens is considered
part of the host defense (Johnson et al., 2001). Thus, one needs to be aware of the
balance between the need for iron for host defense mechanisms and the need for iron to sustain microbial growth. Meanwhile, Bala et al. (1992) reported that copper (Cu) deficiency reduced T cell responses to mitogen. Similarly, Kornegay et al. (1989) showed that high levels of copper seemed to depress the immune response to lysozyme and phytohaemagglutinin (PHA) in weaning pigs.

Additionally, van Heugten and Spears (1997) observed that adding chromium (Cr) to diets of weanling pigs improved growth performance and increased lymphocyte proliferation after adrenocorticotropic hormone (ACTH) administration. This study also showed that supplementation of Cr nicotinate to weanling pigs increases antibody production to sheep red blood cells and decrease antibody production in response to ovalbumin when measured 14 d following immunization (van Heugten and Spears, 1997). However, the effects of applying Cr in the weaning pigs’ diet on immune responses are not consistent. van de Ligt et al. (2002) reported that Cr supplementation in weaning pig’s diet did not significantly affect growth performance and total IgG and IgM concentration in the serum (P > 0.15).

2.2.3 Lipopolysaccharide

Lipopolysaccharides (LPS), consisting of a lipid and a polysaccharide, are found in the outer membrane of gram-negative bacteria and act as endotoxins that trigger strong immune responses in animals (van Heugten and Spears, 1997; Webel et al., 1997). After encountering microorganisms or their secreted substrate, such as LPS or lipoteichoic acid, the leukocytes release cytokines and consequently activate the lymphocytes and complement system, which result in triggered different immune responses and causes inflammation (Cohen, 2002). This inflammation results in
reduced food intake, inactivity, and fever (Johnson and von Borell, 1994; Warren et al., 1997).

The proinflammatory cytokines, e.g., tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), as well as cortisol, are elevated in plasma in response to peripheral LPS injection in weaned pigs. Webel et al. (1997) showed that injecting 5 μg/kg LPS in weaning pigs causes a 10-fold increase in plasma TNF-α level at 2 hr post-injection, 200-fold increase in plasma IL-6 level at 4 hr post-injection, and 10-fold plasma cortisol level at 4 hr post-injection (Figure 2.2). Moreover, plasma urea nitrogen (PUN) also increased 2 to 3-fold at 8 and 12 hr after injection, which indicated that skeletal muscle protein may be degraded due to immune responses. This research also showed that the responses of injecting 0.5 μg/kg LPS in weaning pigs is not as profound as administrating 5 μg/kg LPS.

![Figure 2.2](image.png)

Figure 2.2. The time course of elevated plasma levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), cortisol, and plasma urea nitrogen (PUN) following intraperitoneal injection of LPS (5 mg/kg BW) (Webel et al., 1997).
Overall, pigs with low immunocompetence increase the cost of the conventional swine production system. Optimal dietary strategies may improve the immunocompetence and thus be beneficial for the pig producer.

2.3 Effects of mannan oligosaccharide supplementation as feed additive

2.3.1 Function and mechanism

Most enterobacteria have a specific organelle in the form of type 1 fimbriae that mediates the mannose-specific binding to the intestine wall (Firon et al., 1983). After adhering to the mucosa surface, the pathogens will then proliferate and colonize in the intestine. The attached bacteria can regulate the gene program in intestinal epithelial cells and thus influence the expression of epithelial cell products that subsequently alter the physiological and biochemical function of the intestinal barrier (Kelly, 2004) which suppresses growth performance and immune competence and may lead to diseases and diarrhea.

Mannan oligosaccharides (MOS) are complex sugars that consist mainly of mannose that are found mostly in the cell wall of yeast and plants. The main components of the cell walls are glycoproteins with minor polysaccharides that include cellulose, galactan, and mannan. The polysaccharides that bind to the polysaccharide binding domains of the proteins prevent the protein from being degraded by the proteolytic enzyme, and then strengthen the cell wall (Flores et al., 2000). Yeast mannans have a better binding ability to bind E. coli and Salmonella than other plant mannans due to their specific carbohydrate linkages (Newman, 2006).

MOS was first introduced as an animal feed additive in 1993 (Hooge, 2003); it is not degraded by digestive enzymes, thus it can pass through the digestive tract
Pathogens, which normally adhere to mannans on the mucosal surface of the intestine, may instead bind to the mannan component of yeast products, thus block the bacterial conjugation (Newman, 2006). And if they do, then the pathogens will be flushed from the intestinal tract rather than colonize in the intestine (Newman, 1994). Many studies showed that *E. coli*, which has mannose-specific, lectin-like adhesion protein, will not adhere to mammalian cells when mannose is presented (Salit and Gotschilich, 1977; Ofek and Beachey, 1978).

MOS not only suppresses the attachment of the pathogen to the intestinal wall, but also eliminates those already colonized in the tract (Newman, 1994). Pluske et al. (1997) summarized that pathogens only cause disease when they are colonized and infect the epithelial cell of the intestine. Thus, reduced pathogen colonization results in a healthy gastrointestinal tract and enhanced digestibility, which consequently leads to improved growth performance of the animals. Additionally, the unattached pathogen can also be used as attenuated antigens that are presented to immune cells, thus triggering the immunoreactions (Kocher, 2004). The following section will discuss the effect of feeding MOS on growth performance and immune competence.

2.3.2 Effects of MOS on growth performance

There are many reports showing the benefit of dietary MOS supplements on growth performance of various species. A review paper from Hooge (2006) found that adding a MOS product into the milk replacer (4 g per head per day) that is fed to calves along with starter feed significantly improved the calf body weight gain by 15% and starter feed intake by 10% compared to those calves that received no MOS.
Similarly, MOS also shows effects in other animals. Dietary MOS improved the gain and feed conversion when fed to poultry (Parks et al., 2001; Hooge, 2009; Sim et al. 2004; Hooge, 2003). Mourão et al. (2006) found that MOS supplementation in growing rabbit diets had no effect on growth, but resulted in significantly longer villi in the ileum (510 vs. 403 μm, P = 0.002), which suggested that MOS improved the gut health of the growing rabbits. Understanding the benefit of MOS in these animals helps to explain the potential functions of MOS in pigs.

In pigs, a recent review paper that evaluated all known MOS results with one particular product (Bio-MOS; Alltech Biotechnology Co.) indicated an improvement in weight gain and feed conversion ratio when MOS was introduced into the weanling pig diet (Miguel et al., 2002). The meta-analysis done by Miguel et al. (2002) showed that growth rate, feed intake, and feed efficiency of nursery pigs were significantly improved with MOS supplement (4.2%, P < 0.05; 2.1%, P < 0.05; and -2.2 %, P < 0.05; respectively). The study of LeMieux et al. (2003) showed that MOS improved the performance of nursery pigs when the diet included antibiotic and no excess dietary Zn. Davis et al. (2002) also found there was a positive effect on nursery growth performance from feeding MOS when the diet did not contain excessive levels of Cu.

MOS has been investigated as an alternative to antibiotic supplementation to animals. Rozeboom et al. (2005) examined the effect of MOS on growth performance of weaning pigs and found that MOS improved the growth rate when compared to the control diet that had no antibiotic included (ADG: 394 vs. 368 g, P < 0.05; ADFI: 679 vs. 661 g, P > 0.05). However, the growth rate of MOS treatment was numerically smaller, though not significantly different, than that of the treatment with antibiotics (ADG: 394 vs. 406 g; ADFI: 679 vs. 703 g, P value not provided). These results
indicated that MOS products have the potential to reduce or replace the use of antibiotics.

2.3.3 Effects of MOS on immune responses

Dietary MOS supplementation affects not only growth performance but also the immune systems. Savage et al. (1996) found that concentrations of both blood and bile IgG and IgA were significantly increased in turkeys that were fed MOS. In broiler breeder diets, the addition of MOS increased the antibody response to infectious bursal disease viruses (IBDV) and also increased IBDVI antibody titers in the breeders and progeny (P < 0.05; Shashidhara and Devegowda 2003). The addition of 10 g MOS/ day to the diet of 40 dairy cows resulted in numerically greater serum Ig levels in calves 24-hours after calving than in the calves of unsupplemented cows (Franklin et al., 2005).

MOS has the ability to change the microbial profile and modulate immune function in swine. Davis et al. (2004) observed a greater proportion of blood lymphocytes and decreased blood neutrophils when pigs were fed MOS. White et al. (2002) found that adding MOS to the diet reduced coliform numbers on gut tissue and in digesta from the duodenum, jejunum, cecum, and colon (P < 0.05). Similarly, MOS-fed pigs had lower enterobacteria population compared with control pigs (P < 0.05; Castillo et al., 2008). Spring and Privulescu (1998) reported that dietary supplemental MOS increased serum IgG concentration from 200 to 916 mg/dL (P value not provided) and IgA concentration from 163 to 364 mg/dL (P < 0.05) in germ-free pigs. This research also showed that dietary MOS supplement increased lymphoblast transformation and macrophage activity, whereas, this effect is more
profound in conventional pigs than in germ-free pigs. Pettigrew (2006) observed that MOS changes the microbial population in the digestive tract by reducing the diversity of the population at 21 days after weaning. Those results imply that MOS supplementation has the potential to reduce the outbreak of post-weaning diarrhea by lowering the unfavorable microorganisms.

Castillo et al. (2008) showed that MOS improves gut health by increasing the ratio of lactobacilli:enterobacteria (1.57 vs. 0.91; P < 0.05), which is routinely used as an indicator of gut health, with an increase in the ratio considered beneficial for gut health. The same study also showed that the villus height:crypt depth ratio was higher in MOS-fed pigs. Moreover, Mourão et al. (2006) showed that MOS supplementation results in longer villi in the ileum (P = 0.002). Shorter villi and deeper crypt reduce absorption but increase secretion, which indicates more substrate for pathogen to grow and proliferate. Hence, the results of Castillo et al. (2008) and Mourão et al. (2006) suggested that MOS improved the gut health by changing the intestinal microflora profile and improving intestinal morphology.

2.3.4. Effects of MOS on sows’ reproductive performance

It is important to improve the nutrient intake of a sow during late gestation and through lactation because sows need enough energy to provide for the growth of the fetus. Improving nutrient intake is also necessary for the milk production, the energy for recovering from parturition, and the nutrients for piglet growth. There is no direct evidence that MOS can increase the nutrient absorption, but there is much indirect evidence to show that MOS has the potential to increase growth.
The review paper of Pettigrew et al. (2005) concluded from 5 reports that adding MOS to a sow’s diet 2-3 weeks before parturition and through the lactation period significantly increased the piglets’ average daily gain and subsequent weaning weight, but had no effects on sow weight change or litter size. In addition, Maxwell et al. (2003) reported that dietary MOS supplementation decreased the number of days between weaning and when estrus is exhibited. Medal et al. (2004) showed that adding MOS to the sow diet did not affect the pre-weaning mortality; however, O’Quinn et al. (2001) and Funderburg (2002) showed that pre-weaning mortality was reduced in MOS-fed sows.

The immune system of a neonate is not fully developed, thus the transferred antigen from the sow will be one of the major defenses against pathogens. Several reports have shown that colostrum from MOS-treated sows have numerically higher concentrations of IgG, IgA, and IgM (Newman and Newman, 2001; O’Quinn et al., 2001). Similarly, Funderburke (2002) showed that MOS supplementation to sow diet improved the colostral IgA (P < 0.10), IgG (P < 0.01), and IgM (P < 0.05) concentration.

The intestine of a newborn animal is considered sterile, however soon after birth the intestine is colonized by the gut bacteria from birth canal, teat, sow feces, and the environment of the farrowing pen. Therefore, it is important to reduce the possibility of the neonates being infected by pathogens. Spring and Geliot (2003) observed that adding MOS to the gestation diet 8 weeks prior to farrowing reduced the number of coliforms and C. Perfringens in the feces of the sow during farrowing.
2.4 Conclusion

In swine production systems, reproductive performance is a major factor that influences profits. Meanwhile, dead pigs, specific facilities for weak pigs, as well as injectable antibiotics for ill pigs are extra costs for the pig farm owner. Antibiotics have been used in animal feed as growth promoters and to improve health for past decades. However, the potential development of antibiotic resistance within bacterial populations and residual antibiotics in animal products such as meat and milk has grown in the public concern. Thus, it is necessary to find alternative feed additives that can also improve growth and efficiency of swine production and to modulate the pig’s natural ability to fight diseases. MOS-containing products seem to be one of the possible alternatives based on the current literature.

Therefore, the objective of the studies herein is to more fully evaluate the effect of dietary MOS supplementation on the performances of sow reproduction, neonatal pig growth and health, and consequently growth and health during the nursery period.
Chapter 3. Effect of supplying a mannan oligosaccharide (MOS) containing product to pig diets on response to an immune challenge

3.1. Introduction

Weaning can be a challenge for many young animals because they are removed from their mother (psychological stress), perhaps regrouped with new pen mates (social and disease stress), and their diet is changed from liquid milk to solid feed. These stressful events often occur at a time when their active immunity is not yet mature. Many pathogenic bacteria have Type I fimbria that attach to mannose on the surface of intestinal cells, which is then followed by colonization and development of gastrointestinal disease in the animal (Newman and Spring, 2004).

Mannan oligosaccharides (MOS) are complex sugars based on mannose. Several MOS products have been developed with the view of their potential to modify overall intestinal well being. Pathogens which normally adhere to mannans on the mucosal surface of the intestine may instead bind to the mannan component of products; thus, the pathogens will be flushed from the intestinal tract rather than colonize in the intestine. Improving the overall intestinal health by removing potential pathogens indicates a greater capacity to cope with potential diseases and may ultimately lead to better health and better growth performance (Rozeboom et al., 2005). The objective of this experiment was to evaluate a modified yeast culture feed additive containing MOS on several measures of performance, health, and immune response when young pigs were challenged with lipopolysaccharide (LPS) to simulate the stress of a disease challenge.
3.2. Experimental procedures

This experiment was carried out in environmentally controlled rooms at the University of Kentucky. The experiment was conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

3.2.1 Animals and dietary treatments

A total of 60 crossbred weaned piglets (18-22 days of age) with an average initial body weight of 6.0 ± 0.96 kg were assigned to 4 dietary treatments with 3 pigs/pen (2 barrows and 1 gilt). Treatments were: 1) a control diet fortified to meet NRC (1998) nutrient requirements, 2) the control diet with a low level of MOS product [0.2% for weeks 1-2 and 0.1% for weeks 3-4], 3) the control diet with a high level of MOS product [0.4% for weeks 1-2 and 0.2% for weeks 3-4], and 4) the control diet with 0.25% antibiotic [Mecadox® 10; Phibro Animal Health, Ridgefield Park, NJ; provided 50 g/ton of carbadox]. An enzymatically hydrolyzed yeast and yeast culture product (Cel-Can®; Vi-COR, Mason City IA) containing MOS was the product used. There were 8 pens fed Treatment 1 and 4 pens for the other treatments. Pigs were provided ad libitum access to feed and water (basal diet compositions are provided in Table 3.1). The room temperature was adjusted weekly to maintain it within the thermoneutral zone of the piglets.

3.2.2 Experimental diets

The diets were based primarily on corn, soybean meal, and dried whey which were calculated to contain 3,404 kcal/g ME, 21.92 % CP, and 1.38% Lys for Week 1 and 2; and 3,316 kcal/g ME, 20.80% CP, and 1.21% Lys for Week 3 to 5 (Table 3.1). Minerals and vitamins were added to meet or exceed NRC (1998); antioxidants were
also included in the diets to elongate shelf life of feeds. The treatment diets were made by adding the MOS product to the basal diet by replacing corn.

3.2.3 *Lipopolysaccharide challenge*

After 4 wk of growth performance evaluation, 1 barrow and 1 gilt were selected from each pen for dietary treatments 1-3 for continuance with assessment of immune competence. For the purposes of immune competence assessment, each pig received an intraperitoneal injection of 5 mL phosphate buffered saline (PBS) or LPS (50 μg/kg body weight) suspended in PBS. The pigs were fasted for a 12 h period prior to the LPS and PBS injections to prevent vomiting and for a better estimate of feed intake in response to the injections.

The pigs were injected in the peritoneal cavity 3 nipples down from the caudal end almost lateral to the umbilical region on the left side of the pig. Suspended by the hind legs, each pig was administered the previously determined injection. Starting at 0600 h, a pen consisting of 2 pigs was injected every 15 min until the completion of all 4 pens within the block.

The LPS (serotype O111:B4; L-2630; Sigma, St. Louis, MO) was prepared using sanitized PBS solution (P-4417; Sigma, St. Louis, MO) to make 0.5 mg/mL solution. After the solution was made, it was stored at 4 °C for use the next day. Pigs were weighted before injection to determine the volume of LPS solution to deliver 50 μg/kg body weight in PBS filled up to a final volume of 5mL. The 4 immune treatments were: 1) control diet pigs with PBS injection; 2) control diet pigs with LPS injection; 3) low level MOS pigs with LPS injection; and 4) high level MOS pigs with LPS injection.
Table 3.1. Percentage composition of the basal diet (as-fed basis)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Week 1-2 (phase 1)</th>
<th>Week 3-4 (phase 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, ground, yellow</td>
<td>49.195</td>
<td>59.855</td>
</tr>
<tr>
<td>Dehulled soybean meal, 48% CP</td>
<td>28.000</td>
<td>28.000</td>
</tr>
<tr>
<td>Dried whey</td>
<td>10.000</td>
<td>8.000</td>
</tr>
<tr>
<td>Spray dried animal plasma</td>
<td>1.500</td>
<td>---</td>
</tr>
<tr>
<td>Fish meal, menhaden</td>
<td>3.000</td>
<td>2.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.000</td>
<td>---</td>
</tr>
<tr>
<td>Choice white grease</td>
<td>2.000</td>
<td>---</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>---</td>
<td>0.030</td>
</tr>
<tr>
<td>L-Lysine·HCl</td>
<td>0.060</td>
<td>0.020</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.900</td>
<td>0.750</td>
</tr>
<tr>
<td>Limestone, ground</td>
<td>0.750</td>
<td>0.750</td>
</tr>
<tr>
<td>Salt, plain</td>
<td>0.400</td>
<td>0.400</td>
</tr>
<tr>
<td>Trace-mineral mix&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.075</td>
<td>0.075</td>
</tr>
<tr>
<td>Vitamin mix&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>Santoquin&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.020</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Calculated nutrient composition

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Week 1-2 (phase 1)</th>
<th>Week 3-4 (phase 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME, kcal/g</td>
<td>3,404</td>
<td>3,316</td>
</tr>
<tr>
<td>CP, %</td>
<td>21.62</td>
<td>20.80</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.38</td>
<td>1.21</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.81</td>
<td>0.71</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.68</td>
<td>0.62</td>
</tr>
</tbody>
</table>

<sup>1</sup> Provided (per kilogram of final diet): 150 mg Zn (ZnO); 120 mg Fe (FeSO₄·H₂O); 12 mg Cu (CuSO₄·5H₂O); 45 mg Mn (MnO); 1.5 mg I (CaI₂); 0.3 mg Se (Na₂SeO₃), 12.375 mg Ca (CaCO₃).

<sup>2</sup> Provided (per kilogram of final diet): 6,600 IU of vitamin A, 880 IU of vitamin D₃, 44 IU of vitamin E, 19.3 mg of vitamin K (as Menadione Sodium Bisulfite Complex), 8.8 mg of riboflavin, 22 mg of d-pantothenic acid, 44 mg of niacin, 33 μg of vitamin B₁₂, 220 μg of d-biotin, and 1320 μg of folic acid.

<sup>3</sup> Supplied 130 mg ethoxyquin per kg of basal diet.
3.2.4 Data and sample collection

During the first 4 weeks of growth, pig weight and feed disappearance from each feeder were obtained weekly. For immune competence, body weight, feed disappearance, rectal temperature, respiration rate were measured before injection and every 2 hr after injection for 12 hr. Body weight and feed intake continued to be measured every 24 hr until 1 week post injection. Blood samples were taken before injection and at 2, 4, 8, 12, and 24 hr after injection via the jugular vein from pigs using a V-trough. The blood samples were then centrifuged at 1390 x g at 4 ºC for 20 min to obtain serum. These samples were stored at -20 ºC until needed for analysis of serum cortisol and tumor necrosis factor (TNF-α; a cytokine that is sometimes involved in the fever response).

3.2.5 Laboratory analysis

The frozen serum samples were thawed at 4 ºC for 2 hours before further chemical analysis. The cortisol level in the serum samples were determined by using Coat-A-Count Cortisol Kit (Diagnostic Products Corporation, Los Angeles, CA) for radioimmunoassay (Appendix I). The TNF-α level in the serum samples were determined by using a TNF-α ELISA kit (Pierce Endogen, Rockford, IL) according to assay kit instructions (Appendix II).

3.2.6 Statistical analysis

All data were analyzed as a randomized complete block design with pen as the experimental unit and blocks that were based on initial body weight. Analysis of variance was performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).
Immune growth data were also analyzed using GLM procedure with BW at injection as a covariate. Data obtained from the immune response, including rectal temperature, respiration rate, and serum cortisol and TNF-α level, was also analyzed by the MIXED procedure of SAS to examine time effects.

3.3. Results

3.3.1. Growth performance

There was no significant difference (P > 0.10) between MOS treatments and control treatment on body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed:gain ratio during first 4 weeks of the experiment, except feed:gain ratio on Week 2 and Phase 1 (weekly performance provided in Table 3.2). In Week 2, pigs in the MOS treatments had higher feed:gain ratio compared with those in control treatment (1.27 vs. 1.14, P < 0.05); in Phase 1, pigs in the MOS treatments had higher feed:gain ratio compared with those in control treatment (1.27 vs. 1.16, P = 0.05).

The weekly body weight of the pigs that received antibiotic was 3 to 5% heavier than that of control pigs. The weekly and overall reports of ADG and ADFI were also increased 5 to 7% and 3 to 9%, respectively. These results led to approximately 2% reduction of feed:gain ratio. However, according to the statistic analysis, none of these differences were significant (P > 0.14).

Additionally, compared to the pooled MOS treatments, antibiotic-treated pigs had greater BW at week 2 (10.63 vs. 10.03 kg, P < 0.05), but no other difference between antibiotic treatment and MOS treatment on ADG, ADFI, and feed:gain ratio was detected (P > 0.10).
### Table 3.2. Effects of dietary MOS supplementation on growth performance of weaned pigs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>MOS low</th>
<th>MOS high</th>
<th>Antibiotic</th>
<th>SEM</th>
<th>MOS effect</th>
<th>MOS Linear</th>
<th>MOS Quad</th>
<th>Anti effect</th>
<th>Anti vs. MOS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>5.99</td>
<td>6.00</td>
<td>5.97</td>
<td>6.01</td>
<td>0.02</td>
<td>0.69</td>
<td>0.38</td>
<td>0.47</td>
<td>0.45</td>
<td>0.29</td>
</tr>
<tr>
<td>Week 1</td>
<td>7.22</td>
<td>7.08</td>
<td>7.16</td>
<td>7.42</td>
<td>0.16</td>
<td>0.55</td>
<td>0.76</td>
<td>0.59</td>
<td>0.32</td>
<td>0.15</td>
</tr>
<tr>
<td>Week 2</td>
<td>10.29</td>
<td>10.00</td>
<td>10.06</td>
<td>10.63</td>
<td>0.22</td>
<td>0.26</td>
<td>0.41</td>
<td>0.52</td>
<td>0.24</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Week 3</td>
<td>13.48</td>
<td>13.34</td>
<td>13.25</td>
<td>14.27</td>
<td>0.45</td>
<td>0.68</td>
<td>0.67</td>
<td>0.96</td>
<td>0.17</td>
<td><strong>0.10</strong></td>
</tr>
<tr>
<td>Week 4</td>
<td>17.69</td>
<td>17.43</td>
<td>17.79</td>
<td>18.42</td>
<td>0.52</td>
<td>0.88</td>
<td>0.87</td>
<td>0.62</td>
<td>0.27</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Average daily gain, ADG (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>175</td>
<td>156</td>
<td>170</td>
<td>201</td>
<td>24.0</td>
<td>0.60</td>
<td>0.85</td>
<td>0.55</td>
<td>0.39</td>
<td>0.21</td>
</tr>
<tr>
<td>Week 2</td>
<td>439</td>
<td>417</td>
<td>414</td>
<td>459</td>
<td>20.1</td>
<td>0.26</td>
<td>0.33</td>
<td>0.69</td>
<td>0.43</td>
<td>0.10</td>
</tr>
<tr>
<td>Week 3</td>
<td>456</td>
<td>477</td>
<td>456</td>
<td>521</td>
<td>40.6</td>
<td>0.80</td>
<td>0.99</td>
<td>0.66</td>
<td>0.21</td>
<td>0.29</td>
</tr>
<tr>
<td>Week 4</td>
<td>601</td>
<td>584</td>
<td>649</td>
<td>592</td>
<td>27.4</td>
<td>0.56</td>
<td>0.17</td>
<td>0.23</td>
<td>0.80</td>
<td>0.48</td>
</tr>
<tr>
<td>Phase 1</td>
<td>307</td>
<td>286</td>
<td>292</td>
<td>330</td>
<td>16.2</td>
<td>0.28</td>
<td>0.45</td>
<td>0.49</td>
<td>0.27</td>
<td>0.06</td>
</tr>
<tr>
<td>Phase 2</td>
<td>528</td>
<td>531</td>
<td>552</td>
<td>557</td>
<td>23.9</td>
<td>0.59</td>
<td>0.42</td>
<td>0.73</td>
<td>0.35</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>418</td>
<td>409</td>
<td>422</td>
<td>443</td>
<td>18.8</td>
<td>0.90</td>
<td>0.85</td>
<td>0.61</td>
<td>0.28</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Average daily feed intake, ADFI (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>212</td>
<td>193</td>
<td>221</td>
<td>226</td>
<td>19.1</td>
<td>0.81</td>
<td>0.69</td>
<td>0.31</td>
<td>0.56</td>
<td>0.44</td>
</tr>
<tr>
<td>Week 2</td>
<td>508</td>
<td>529</td>
<td>518</td>
<td>558</td>
<td>26.0</td>
<td>0.55</td>
<td>0.74</td>
<td>0.61</td>
<td>0.14</td>
<td>0.30</td>
</tr>
<tr>
<td>Week 3</td>
<td>926</td>
<td>918</td>
<td>877</td>
<td>970</td>
<td>51.3</td>
<td>0.59</td>
<td>0.45</td>
<td>0.78</td>
<td>0.49</td>
<td>0.27</td>
</tr>
<tr>
<td>Week 4</td>
<td>954</td>
<td>919</td>
<td>1013</td>
<td>965</td>
<td>36.0</td>
<td>0.73</td>
<td>0.20</td>
<td>0.15</td>
<td>0.78</td>
<td>1.00</td>
</tr>
<tr>
<td>Phase 1</td>
<td>360</td>
<td>361</td>
<td>370</td>
<td>392</td>
<td>17.5</td>
<td>0.75</td>
<td>0.64</td>
<td>0.85</td>
<td>0.16</td>
<td>0.24</td>
</tr>
<tr>
<td>Phase 2</td>
<td>940</td>
<td>919</td>
<td>945</td>
<td>968</td>
<td>35.9</td>
<td>0.82</td>
<td>0.90</td>
<td>0.58</td>
<td>0.53</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>650</td>
<td>640</td>
<td>657</td>
<td>680</td>
<td>22.3</td>
<td>0.95</td>
<td>0.78</td>
<td>0.60</td>
<td>0.29</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Feed/gain ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>1.24</td>
<td>1.26</td>
<td>1.32</td>
<td>1.15</td>
<td>0.09</td>
<td>0.57</td>
<td>0.46</td>
<td>0.84</td>
<td>0.44</td>
<td>0.22</td>
</tr>
<tr>
<td>Week 2</td>
<td>1.14</td>
<td>1.27</td>
<td>1.26</td>
<td>1.22</td>
<td>0.06</td>
<td><strong>0.04</strong></td>
<td>0.09</td>
<td>0.31</td>
<td>0.25</td>
<td>0.51</td>
</tr>
<tr>
<td>Week 3</td>
<td>2.10</td>
<td>1.97</td>
<td>1.94</td>
<td>1.95</td>
<td>0.14</td>
<td>0.32</td>
<td>0.36</td>
<td>0.77</td>
<td>0.40</td>
<td>0.98</td>
</tr>
<tr>
<td>Week 4</td>
<td>1.59</td>
<td>1.57</td>
<td>1.57</td>
<td>1.63</td>
<td>0.04</td>
<td>0.55</td>
<td>0.60</td>
<td>0.85</td>
<td>0.39</td>
<td>0.19</td>
</tr>
<tr>
<td>Phase 1</td>
<td>1.16</td>
<td>1.26</td>
<td>1.27</td>
<td>1.20</td>
<td>0.05</td>
<td><strong>0.05</strong></td>
<td>0.09</td>
<td>0.42</td>
<td>0.53</td>
<td>0.28</td>
</tr>
<tr>
<td>Phase 2</td>
<td>1.80</td>
<td>1.74</td>
<td>1.72</td>
<td>1.75</td>
<td>0.06</td>
<td>0.21</td>
<td>0.22</td>
<td>0.80</td>
<td>0.49</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1.57</td>
<td>1.58</td>
<td>1.56</td>
<td>1.55</td>
<td>0.05</td>
<td>0.98</td>
<td>0.88</td>
<td>0.85</td>
<td>0.69</td>
<td>0.71</td>
</tr>
</tbody>
</table>

1Each means represents 4 pens of 3 pigs/pen.

2Low MOS treatment was the control diet with 0.2 % MOS for Phase 1 and 0.1 % MOS for Phase 2; High MOS treatment was the control diet with 0.4 % MOS for Phase 1 and 0.2 % MOS for Phase 2; Antibiotic treatment was the control diet with 0.25 % antibiotic (as Mecadox) for both Phase 1 and Phase 2.

3P-value of MOS effect was the pooled MOS group compared with the control group; MOS linear and quadratic P-value compared the MOS-fed groups and the control group; P-value of antibiotic was the antibiotic group compared with the control group; P-value of Anti vs. MOS is the MOS groups compared with the antibiotic group.
3.3.2. Stress responses

Cumulative growth performance

Differences were observed among treatments in response to the LPS immune challenge. As shown in Table 3.3, Table 3.4 and Figure 3.1, the control pigs challenged with LPS lost weight at 2 hr post-injection while PBS-injected pigs gained weight (-145 vs. 341 g; P < 0.01). The weight loss of the pigs challenged with LPS was not recovered until 12 h post-injection. The LPS-challenged control pigs also had lower cumulative feed intake (CFI) compared to PBS-injected pigs (43 vs. 181 g; P = 0.01; Figure 3.1c). Cumulative weight gain (CWG) or CFI differences were not detectable (P > 0.20) at 48 hr post-injection. MOS supplementation of pigs challenged with LPS had no effect on CWG or CFI compared to LPS-challenged control pigs. The LPS effect was not affected by the initial body weight of the injection (data not shown).
Table 3.3. Effects of dietary MOS supplementation on body weight (kg) of LPS challenged pigs.\textsuperscript{12}

<table>
<thead>
<tr>
<th>Injection:</th>
<th>PBS\textsuperscript{3}</th>
<th>LPS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOS: 0</td>
<td>0</td>
<td>Low</td>
<td>High</td>
<td>LPS\textsuperscript{4}</td>
</tr>
<tr>
<td>Low</td>
<td>15.61</td>
<td>17.62</td>
<td>16.80</td>
<td>16.94</td>
</tr>
<tr>
<td>High</td>
<td>15.95</td>
<td>17.47</td>
<td>16.67</td>
<td>16.86</td>
</tr>
<tr>
<td>Pooled</td>
<td>16.22</td>
<td>17.37</td>
<td>16.41</td>
<td>16.80</td>
</tr>
<tr>
<td>0h</td>
<td>16.19</td>
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<td>20.52</td>
<td>21.02</td>
</tr>
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<td>21.09</td>
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<td>22.52</td>
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</table>

\textsuperscript{1} Each mean represents 4 pens of 2 pigs/pen.
\textsuperscript{2} Treatments in this trial were 1) pigs fed control diet with PBS injection; 2) pigs fed control diet with LPS injection; 3) pigs fed low MOS (1 \%) diet with LPS injection; and 4) pigs fed high MOS (2 \%) diet with LPS injection.
\textsuperscript{3} 1 pen in control treatment was removed from the data set because the 2 pigs in this pen were injected differently.
\textsuperscript{4} Compared PBS group and LPS group without supplemental MOS.
\textsuperscript{5} Low MOS compared the low MOS supplemented LPS-challenged pigs and the LPS-challenged control group; High MOS compared the high MOS supplemented LPS-challenged pigs and the LPS-challenged control group; pooled compared the pooled LPS-challenged MOS-fed pigs with LPS-challenged control pigs.
Table 3.4. Effects of dietary MOS supplementation on growth performance of LPS challenged pigs.12

<table>
<thead>
<tr>
<th>Injection:</th>
<th>PBS3</th>
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<th>SEM</th>
<th>P-value</th>
<th>MOS5</th>
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</thead>
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<td>0</td>
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<td>High</td>
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<td>Cumulative body weight gain, g</td>
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<td></td>
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<tr>
<td>0-2 h</td>
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<td>-125</td>
<td>-72</td>
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</tr>
<tr>
<td>0-4 h</td>
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<td>-425</td>
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<td>1,163</td>
<td>70</td>
<td>-305</td>
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<td>520</td>
<td>238</td>
<td>590</td>
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<td>1,948</td>
<td>1,695</td>
<td>1,998</td>
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<td>2,603</td>
<td>2,585</td>
<td>2,638</td>
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</tr>
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<td>3,265</td>
<td>3,138</td>
<td>3,290</td>
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<tr>
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<td>3,915</td>
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<td>Cumulative feed intake, g</td>
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<tr>
<td>0-2 h</td>
<td>181</td>
<td>43</td>
<td>36</td>
<td>76</td>
<td>27.0</td>
</tr>
<tr>
<td>0-4 h</td>
<td>306</td>
<td>75</td>
<td>54</td>
<td>101</td>
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<td>0-6 h</td>
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<td>1,926</td>
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<td>3,243</td>
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<td>3,161</td>
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</tr>
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</table>

1 Each mean represents 4 pens of 2 pigs/pen.
2 Treatments: 1) control diet with PBS injection; 2) control diet with LPS injection; 3) low MOS (1%) diet with LPS injection; and 4) high MOS (2%) diet with LPS injection.
3 1 pen in control treatment was removed from the data set because the 2 pigs in this pen were injected differently.
4 Compared PBS group and LPS group without supplemental MOS.
5 Low MOS compared the low MOS supplemented LPS-challenged pigs and the LPS-challenged control group; High MOS compared the high MOS supplemented LPS-challenged pigs and the LPS-challenged control group; pooled compared the pooled LPS-challenged MOS-fed pigs with LPS-challenged control pigs.
Figure 3.1. Effects of phosphate buffered saline (PBS) or lipopolysaccharide (LPS) challenge on body weight (a), cumulative BW gain (b), and cumulative feed intake (c). The volume of LPS solution was determined to deliver 50 $\mu$g/kg body weight in PBS filled up to 5mL.
Table 3.5 Effects of dietary MOS supplementation on respiratory rate and rectal temperature of LPS challenged pigs.

<table>
<thead>
<tr>
<th>Injection:</th>
<th>PBS⁵</th>
<th>LPS</th>
<th>SEM</th>
<th>P-value</th>
<th>MOS⁵</th>
<th>LPS¹</th>
<th>MOS²</th>
<th>Pooled</th>
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<td>Low</td>
<td>High</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1h</td>
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<tr>
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<td>67.6</td>
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<td>&lt;.001</td>
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<td>0.01</td>
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<tr>
<td>9h</td>
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<td>48.5</td>
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<tr>
<td>11h</td>
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<td>45.9</td>
<td>49.3</td>
<td>40.1</td>
<td>3.6</td>
<td>0.22</td>
<td>0.51</td>
<td>0.28</td>
</tr>
<tr>
<td>25h</td>
<td>37.2</td>
<td>42.3</td>
<td>49.9</td>
<td>40.8</td>
<td>4.8</td>
<td>0.51</td>
<td>0.28</td>
<td>0.83</td>
</tr>
<tr>
<td>28h</td>
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<td>50.5</td>
<td>43.6</td>
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<td>0.35</td>
<td>0.37</td>
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<tr>
<td>Rectal Temperature, °C</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0h</td>
<td>39.0</td>
<td>39.5</td>
<td>39.3</td>
<td>39.3</td>
<td>0.11</td>
<td>0.03</td>
<td>0.24</td>
<td>0.30</td>
</tr>
<tr>
<td>2h</td>
<td>39.4</td>
<td>40.2</td>
<td>39.9</td>
<td>39.8</td>
<td>0.11</td>
<td>0.001</td>
<td>0.12</td>
<td>0.04</td>
</tr>
<tr>
<td>4h</td>
<td>39.5</td>
<td>40.2</td>
<td>39.4</td>
<td>40.0</td>
<td>0.21</td>
<td>0.03</td>
<td>0.04</td>
<td>0.53</td>
</tr>
<tr>
<td>6h</td>
<td>39.6</td>
<td>39.9</td>
<td>39.6</td>
<td>39.9</td>
<td>0.25</td>
<td>0.22</td>
<td>0.46</td>
<td>0.89</td>
</tr>
<tr>
<td>8h</td>
<td>39.6</td>
<td>39.5</td>
<td>39.8</td>
<td>39.9</td>
<td>0.12</td>
<td>0.84</td>
<td>0.13</td>
<td>0.04</td>
</tr>
<tr>
<td>10h</td>
<td>39.6</td>
<td>39.6</td>
<td>39.9</td>
<td>39.6</td>
<td>0.10</td>
<td>0.49</td>
<td>0.08</td>
<td>0.80</td>
</tr>
<tr>
<td>12h</td>
<td>39.6</td>
<td>39.5</td>
<td>39.8</td>
<td>39.5</td>
<td>0.20</td>
<td>0.94</td>
<td>0.28</td>
<td>0.98</td>
</tr>
<tr>
<td>24h</td>
<td>39.4</td>
<td>39.3</td>
<td>39.6</td>
<td>39.3</td>
<td>0.10</td>
<td>0.36</td>
<td>0.11</td>
<td>0.81</td>
</tr>
</tbody>
</table>

1 Each mean represents 4 pens of 2 pigs/pen.
2 Treatments: 1) control diet with PBS injection; 2) control diet with LPS injection; 3) low MOS (1%) diet with LPS injection; and 4) high MOS (2%) diet with LPS injection.
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4 Compared PBS group and LPS group without supplemental MOS.
5 Low MOS compared the low MOS supplemented LPS-challenged pigs and the LPS-challenged control group; High MOS compared the high MOS supplemented LPS-challenged pigs and the LPS-challenged control group; pooled compared the pooled LPS-challenged MOS-fed pigs with LPS-challenged control pigs.
Respiratory rate and rectal temperature

With regard to whole-body physiological measures, Table 3.5 and Figure 3.2 illustrate that respiratory rate of LPS-injected pigs was higher than PBS-injected pigs at 3 hr post-injection (83 vs. 38 breaths/min; P < 0.01) and was linearly decreased as MOS inclusion was increased in the LPS-injected pigs (83, 68, and 64 breaths/min, respectively; P = 0.01). Rectal temperature (Table 3.5 and Figure 3.2) was higher in the LPS-injected control pigs than in the PBS-injected pigs at 2 hr (40.2 vs. 39.4 ºC; P < 0.01) and at 4 hr (40.2 vs. 39.5 ºC; P < 0.05) which is consistent with the expectations of this challenge model; MOS-fed pigs had a lower rectal temperature than the LPS-controls at 2 hr post-injection (40.2 vs. 39.9 ºC; P < 0.05).
Figure 3.2. Effects of phosphate buffered saline (PBS) or lipopolysaccharide (LPS) challenge on (a) respiratory rate and (b) rectal temperature. The volume of LPS solution was determined to deliver 50 μg/kg body weight in PBS filled up to 5mL.
*Serum cortisol and TNF-α level*

With regard to serum measures, cortisol levels were higher in the LPS-injected control pigs than in the PBS-injected pigs at 2 hr (17.3 vs. 8.6 mcg/dL; P < 0.01) and at 4 hr (22.8 vs. 8.9 mcg/dL; P < 0.01) but were not moderated by MOS supplementation (Table 3.6 and Figure 3.3). The serum TNF-α responses measured at 2 hr post-injection (35, 1925, 2069, and 2833 pg/mL for Treatments 1-4, respectively) revealed an increase due to the LPS injection (P < 0.05) but no reduction in MOS-fed pigs (P > 0.20).

### Table 3.6. Effects of dietary MOS supplementation on serum cortisol level and serum TNF-α level of LPS challenged pigs.12

<table>
<thead>
<tr>
<th>Injection:</th>
<th>PBS3</th>
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<th>SEM</th>
<th>P-value</th>
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<td>0</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td></td>
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<td>4 h</td>
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<td>22.8</td>
<td>22.44</td>
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<tr>
<td></td>
<td>24 h</td>
<td>8.0</td>
<td>7.4</td>
<td>7.15</td>
</tr>
</tbody>
</table>

| Serum cortisol level (µg / dL) | | |
|-------------------------------|---|---|---|---|
| 0 h                           | 10.5| 11.4| 10.13| 12.1| 1.24| 0.63| 0.48| 0.70| 0.84|
| 2 h                           | 8.6 | 17.3| 17.43| 16.3| 2.02| 0.004| 0.96| 0.72| 0.85|
| 4 h                           | 8.9 | 22.8| 22.44| 22.8| 3.80| <.001| 0.90| 0.98| 0.93|
| 8 h                           | 6.9 | 9.2 | 13.46| 11.7| 1.80| 0.33 | 0.13 | 0.35 | 0.16|
| 12 h                          | 6.4 | 8.1 | 8.64 | 6.8 | 1.03| 0.03 | 0.70 | 0.42 | 0.79|
| 24 h                          | 8.0 | 7.4 | 7.15 | 7.1 | 0.61| 0.57 | 0.76 | 0.74 | 0.71|

| Serum TNF-α level (pg/mL) | | |
|---------------------------|---|---|---|---|---|---|---|---|
| 2 h                       | 34.0| 1,162.5| 2069.3| 2,134.1| 597.23| 0.24| 0.31| 0.28| 0.23|

1 Each mean represents 4 pens of 2 pigs/pen.
2 Treatments: 1) control diet with PBS injection; 2) control diet with LPS injection; 3) low MOS (1%) diet with LPS injection; and 4) high MOS (2%) diet with LPS injection.
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Figure 3.3. Effects of phosphate buffered saline (PBS) or lipopolysaccharide (LPS) challenge on (a) serum cortisol level and (b) serum TNF-α level at 2 hr post injection. The volume of LPS solution was determined to deliver 50 μg/kg body weight in PBS filled up to 5mL.
3.4. Discussion

3.4.1. Growth performance

MOS has been reported to block the pathogen colonizing on the intestinal wall thus changing the microorganism population in the gastrointestinal tract (Newman, 1994; Spring et al., 2000). Reduced pathogen colonization indicates a healthy gastrointestinal tract and enhanced digestibility, which consequently lead to improved growth performance of the animals (Pluske et al. 1997). The effect of dietary MOS supplementation has been studied on growth performance of various species, such as calves (Hooge, 2006), poultry (Park et al., 2001; Sim et al., 2004), and rabbits (Mourão et al., 2006). Most of these studies showed that dietary MOS improved their growth rates. However, not all the results are consistent.

Previous studies have reported that MOS supplementation during the nursery period immediately after weaning improves growth rates of piglets. Davis et al. (2004) showed that adding 0.3% MOS in the nursery pigs’ diets (including 0.15 g of neomycin as neomycin sulfate and 0.11 g of oxytetraccline per kilogram of feed) improve weight gain and gain:feed ratio in the first 14 d (P = 0.05 and P = 0.01, respectively) and overall nursery period (P = 0.03 and P = 0.02, respectively). In addition, Davis et al. (2002) found that adding MOS into the nursery pigs’ diet with or without CuSO₄ improved weight gain (P = 0.04) and gain:feed ratio (P = 0.04) of the overall period (d 0-38 post-weaning). A meta-analysis showed that growth rate, feed intake, and feed efficiency of nursery pigs were improved with MOS supplementation from the product Bio-MOS (4.2%, 2.1% and -2.2 %, respectively; Miguel et al., 2002).

However, in the present study, there was no improvement in body weight, ADG, and ADFI with supplementation of MOS. Yet, the feed:gain ratio was
decreased at 2 wk post-weaning (1.24 vs. 1.14, \( P = 0.04 \)) and phase 1 (1.24 vs. 1.16, \( P = 0.05 \)). This result is consistent with Cas tillo et al. (2008). Similarly, LeMieux et al. (2003) reported that MOS improved the performance of nursery pigs when the diet included antibiotic and no excess dietary Zn after d 7 post-weaning. This report suggested that the MOS effect may take at least 1 wk for alternation of the intestinal microflora to take place.

Furthermore, Rozeboom et al. (2005) compared the effect of MOS on growth performance of weaning pigs with antibiotic supplementation and found that MOS improved the growth rate when the control diet included no antibiotic, though the improvement is not as profound as the improvement of adding antibiotic. In the present study, there is no difference between the MOS-fed group and antibiotic-fed group. However, there is no difference between the antibiotic group and control group in this study. This failure to provide a growth response may be related to the cleanliness of the research facilities. Previous evaluations of growth responses to a variety of dietary amendments have illustrated greater responses when the facilities were not as well sanitized (Coffey and Cromwell, 2001; Cromwell, 2001). Thus the failure to see a growth response in this study may have been due to using a different MOS product or to facilities being too clean.

### 3.4.2. Immune challenge

Various reports suggested that MOS enhanced growth performance due to its feature of improving gut health as well as immune system (Newman, 1994; Spring et al., 2000). Castillo et al. (2008) found that supplying MOS in the nursery diet decreased enterobacteria population in the distal jejunum (\( P < 0.05 \)) at 14 d...
post-weaning. Similarly, Mourão et al. (2006) observed lower total bacteria count in ileal ($P < 0.001$) and cecal content ($P = 0.02$) of MOS-fed rabbits at 46 d of age.

In the present study, LPS injection was utilized to mimic the activation of the immune system. Pigs challenged with LPS develop an inflammation, which causes reduced food intake, inactivity, and fever. Johnson and von Borell (1994) observed that pigs’ feed intake is reduced after injecting 50 $\mu$g/kg LPS and is still not recovered after 8 hr post injection, which is consistent with the current study. However, MOS supplementation on pigs challenged with LPS had no effect on cumulative weight gain (CWG) or cumulative feed intake (CFI) compared to LPS-challenged control pigs.

The respiratory rates of LPS-injected pigs are significantly increased at 3 hr post injection ($P < 0.001$) and are not recovered after 7 hr post injection ($P > 0.15$). In addition, rectal temperature is higher in LPS-injected pig than in PBS-injected pigs from 0 to 4 hr post injection ($P < 0.03$) and are recovered at 6 hr post injection ($P > 0.20$). This response is similar to that of Wright et al. (2000), who reported that rectal temperature of pig injected 100 $\mu$g/kg LPS is elevated at 2, 4, 6, 8, and 12 hr after administration ($P < 0.05$). The difference of temperature in LPS-treated pigs and control pigs are not detected after 24 hr post injection (Wright et al., 2000).

Furthermore, Webel et al. (1997) showed that plasma TNF-$\alpha$ and cortisol levels after LPS challenge were both increased at 2 and 4 hr, but returned to control levels at 8 hr after injection. Wright et al. (2000) reported similar results that plasma cortisol was increased in LPS-treated pigs at 2, 4, 6, 8, and 12 hr after LPS injection and that plasma TNF-$\alpha$ was raised at 2 and 4 hr after LPS injection ($P < 0.001$). These results were similar to the current experiment that cortisol levels were higher in the LPS-injected control pigs than in the PBS-injected pigs at 2 hr and at 4 hr ($P < 0.01$).
However, compared to the LPS-challenged control pigs, MOS-supplemented pigs had a lower rectal temperature and respiratory rate during the first 4 hr after injection, which implied that MOS improves some aspect of the immune function of piglets when challenged with LPS. While the rectal temperature and respiratory responses are positive, the mechanism of how these reductions were accomplished is not yet understood. Certainly it was not by an effect on serum cortisol or TNF-α level since there is no difference between MOS-fed or control pigs challenged with LPS.

Overall, nursery pigs challenged with LPS literally reduced growth rate and feed consumption as well as increased body temperature and respiratory rate. They also exhibited elevated plasma cortisol and TNF-α concentration. Although MOS had no effect on growth performance in this experiment, it provides some benefit for weaned pigs during an immune challenge.

3.5. Implication

The present study indicated that dietary MOS supplementation to weaning pigs housed in the sanitation-controlled room may not affect the growth performance during nursery period and after challenge with LPS. However, some inflammatory responses caused by LPS injection are alleviated in MOS-fed pigs. Further research may need to be conducted to evaluate the effect of MOS supplementation on nursery pigs’ growth performance and immune responses in the conventional/commercial environment.
Abstract

Weaning is a challenge for piglets because they are removed from their mother, regrouped with new pen mates, and their diet is changed from liquid milk to solid feed. These stressful events also occur at a time when their active immunity is not yet mature which may inhibit their response to diseases. The objective of this experiment was to evaluate a modified yeast culture feed additive containing MOS, which has been suggested to improve immune responses, in pigs challenged with lipopolysaccharide (LPS). Weaned pigs were assigned to 4 treatments with 3 pigs per pen. The dietary treatments included: 1) control basal diet [n=24], 2) low level MOS [0.2% in phase 1 and 0.1% in phase 2 diet; n=12], 3) high level MOS [0.4% in phase 1 and 0.2% in phase 2 diet; n=12], and 4) the control diet with 0.25% antibiotic [Mecadox, n=12]. After 4 wk of the growth experiment, 32 pigs (2 pigs per pen from Treatment 1 to 3) continued on the diets to examine the response to LPS injection. At 0600 on d 29, each pig received an injection of phosphate buffered saline (PBS; for half of Treatment 1 pigs) or LPS (for all other pigs). At 2 h post-injection, the control pigs challenged with LPS lost weight compared with PBS-injected pigs (-145 vs. 341 g; P < 0.01) and had lower cumulative feed intake (43 vs. 181 g; P = 0.01). Cumulative weight gain (CWG) or CFI differences were not detectable at 48 h post-injection. MOS supplementation had no effect on CWG or CFI. Respiratory rate of LPS-injected control pigs was higher than PBS-injected pigs at 3 h post-injection (83 vs. 38 breath/min; P < 0.01) and was linearly decreased as MOS inclusion was increased in LPS-injected pigs (83, 68, and 64 breaths/min, respectively; P < 0.01). Rectal temperature was higher in the LPS-injected control pigs than in the PBS-injected pigs at 2 h (40.2 vs. 39.4 °C; P < 0.01) and at 4 h (40.2 vs. 39.5 °C; P < 0.01); MOS fed pigs had a lower rectal temperature than the LPS-controls at 2 h.
post-injection (P = 0.03). Serum cortisol values were higher in the LPS-injected control pigs than in the PBS-injected pigs at 2 h (17.3 vs. 8.6 μg/dL; P = 0.03) and at 4 h (22.8 vs. 8.9 μg/dL; P < 0.01) but were not moderated by MOS supplementation. The results showed that LPS challenge affects CWG, CFI, rectal temperature, respiratory rate, and cortisol levels and that dietary MOS supplementation reduces LPS-induced effects on respiratory rate and rectal temperature, but not on CWG, CFI or cortisol levels; thus MOS may provide some benefit for weaned pigs during an immune challenge.
Chapter 4. Effect of supplying a mannan oligosaccharide (MOS) containing product to sow diets on reproductive responses and further effects on nursery pigs

4.1. Introduction

The neonatal intestine is considered as a germ-free environment (Maxwell and Stewart, 1995). After the neonates start eating and are exposed to the atmosphere, many bacteria will enter the intestine and then colonize. If the major colonization is pathogenic bacteria, it may result in the occurrence of diarrhea or other disease. To enhance the young animal’s immune resistance, it is important to improve the quality and immunoglobulin quantity of milks.

Mannan oligosaccharide (MOS) is a complex sugar which consists mainly of mannose. The mannan functions as ligands for the specific structure, type 1 fimbrial, of pathogenic bacteria, such as E. coli, and salmonellae. Once the bacteria “recognize” the mannan on the cells in the intestine, they bind to the cell and then colonize in the intestine, which subsequently may lead to disease (Newman, 1994). If bacteria recognize and bind with the dietary MOS, they may be flushed out of the intestine instead of attaching and colonizing the intestinal wall. Dietary MOS supplementation has been studied for improving the growth performance and immunity of weanling animals (LeMieux et al., 2003; Miguel et al., 2004) and reproductive performance of sow (Maxwell et al., 2003; Newman and Newman, 2001).

The aim of this experiment was to determine the effect of supplying MOS to sow diets on reproductive performance and milk quality and subsequent growth performance of piglets fed with or without MOS.
4.2. Experimental procedures

This experiment was carried out in environmentally controlled rooms at the University of Kentucky Swine Unit. The experiment was conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

4.2.1 Experiment 1

Animal and treatments

A total of 28 sows (Yorkshire or Landrace × Yorkshire) with an average parity of 1.63 ± 0.92 were assigned to 2 dietary treatments, including 1) control diet that met NRC [1998] nutrient requirements and 2) the control diet with a MOS product [0.2% for both gestation and lactation diets]. A modified yeast culture feed additive (Celmanax®; Vi-COR, Mason City IA) was the product used in this experiment. Celmanax is a unique enzymatically hydrolyzed yeast (EHY) product made of yeast culture and hydrolyzed yeast cell wall. It contains yeast culture and other complex carbohydrates like D-galactosamine, glucosamine, beta glucans and mannan oligosaccharides.

Sows were allotted to treatment based on parity, breed and breeding weight and were housed in individual gestation stalls (0.61 m x 2.13 m) with the rear 0.66 m slatted with concrete slats. Individual floor feeding at a level of 1.8 kg/d was maintained throughout gestation and water was available from water nipples on an ad libitum basis. The experiment started 14 days before the expected farrowing date, approximately day 102 of gestation.

On approximately d 108 of gestation, sows were moved to a temperature-regulated farrowing facility and placed in farrowing stalls (0.61 m x 2.13 m)
m) with plastic-coated welded wire flooring. Diets were changed to lactation diets and were fed on an ad libitum basis until farrowing. Sows were provided 3.2 kg of lactation diet on the day of farrowing, and then increased 0.9 kg until the sow started leaving feed in the feeder. On the day of weaning, approximately d 19 of lactation, sows were returned to the breeding facility for detection of estrous and rebreeding. Gestation room temperature and farrowing/lactation room temperature and humidity were recorded daily.

**Experimental diets**

The diets were based primarily on corn and soybean meal and were calculated to contain 3,364 kcal/g ME, 12.62 % CP, and 0.58% Lysine for the gestation diet; 3,408 kcal/g ME, 17.65% CP, and 1.01% lysine for the lactation diet (Table 4.1). Minerals and vitamins were added to meet or exceed NRC (1998). The treatment diets were made by adding the MOS product to the basal diet by replacing of corn.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gestation</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>81.54</td>
<td>67.82</td>
</tr>
<tr>
<td>Soybean meal, 48% CP</td>
<td>12.31</td>
<td>25.15</td>
</tr>
<tr>
<td>Grease</td>
<td>2.00</td>
<td>3.00</td>
</tr>
<tr>
<td>L-Lysine·HCl</td>
<td>0.00</td>
<td>0.10</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.63</td>
<td>2.36</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.69</td>
<td>0.74</td>
</tr>
<tr>
<td>Salt</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Mineral mix&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Vitamin mix&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Choline chloride – 60%</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Chromax&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Calculated nutrient composition

<table>
<thead>
<tr>
<th></th>
<th>Gestation</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME, kcal/g</td>
<td>3,364</td>
<td>3,408</td>
</tr>
<tr>
<td>CP, %</td>
<td>12.62</td>
<td>17.65</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>0.58</td>
<td>1.01</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.89</td>
<td>0.89</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.80</td>
<td>0.80</td>
</tr>
</tbody>
</table>

<sup>1</sup> Provided (per kilogram of final diet): 160 mg Zn (ZnO); 128 mg Fe (FeSO₄·H₂O); 12.8 mg Cu (CuSO₄·5H₂O); 48 mg Mn (MnO); 1.6 mg I (CaI₂), 0.32 mg Se (Na₂SeO₃), 13.2 mg Ca (CaCO₃).

<sup>2</sup> Provided (per kilogram of final diet): 3,300 IU of vitamin A, 440 IU of vitamin D₃, 22 IU of vitamin E, 9.67 mg of vitamin K (as Menadione Sodium Bifulfite Complex), 4.4 mg of riboflavin, 11 mg of d-pantothenic acid, 22 mg of niacin, 16.5 μg of vitamin B₁₂, 110 μg of d-biotin, and 660 μg of folic acid.

<sup>3</sup> Chromax (Prince Agri Products, Inc., Quincy, IL) provided 200 ppb Cr as chromium tripicolinate.
Data and sample collection

Sow feed consumption during lactation was recorded daily. Sows weight were obtained on breeding day, pre-feeding (gestation d 101–102), pre-farrowing (gestation d 111-113), within 24 h post-farrowing, and at weaning. The number of pigs born (alive and dead) and the birth weight of each pig were recorded within 24 h of farrowing. In addition, pigs received ear-notches, clipping of needle teeth, and injection with 100 mg Fe as Fe dextran on the same day. Some of the piglets were transferred to other litters within treatment within 3 days after birth to balance the litter size. No transferred piglet died during the experiment. Male piglets were castrated at 10-13 days of age. Creep feed was not offered, but access to the sow’s feed was not restricted. Individual pig weaning weights were also recorded.

Blood samples from the sows were collected by jugular venipuncture at pre-feeding, pre-farrowing (d111-113 of gestation), early lactation (d 4-6 of lactation) and late lactation (d 15-17 of lactation). Colostrum samples were collected within 24 hr of farrowing. Milk samples were collected to represent early lactation and late lactation respectively. Colostrum or milk was hand-expressed from the third and forth functional teats of both sides for a total collection of approximately 50 mL. A 1 mL oxytocin (20 USP) injection was administered IM in the neck and shoulder area to facilitate collection of the colostrum and milk samples. Serum samples from 5 piglets in the mid-weight range of each litter were collected by jugular venipuncture using a V-trough during early lactation and late lactation. Serum was pooled from those 5 piglets in the same litter in same ratio (approximately 0.1 mL from each piglet); the remaining serum was kept individually as backup.
4.2.2 Experiment 2

Animals, treatments, and diets

A total of 104 weaned piglets from 7 sows on each treatment in Experiment 1 were assigned to 2 diets within the litters, including 1) control diet that meet NRC [1998] nutrient requirements and 2) the control diet with MOS product [0.2% for both Phase 1 and Phase 2 diets]. As each litter was weaned, equal number of barrows and gilts were assigned to each nursery diet (extra pigs were not used in the nursery experiment). This resulted in a 2 × 2 factorial arrangement with the experimental treatments as 1) control sow diet with control nursery diet, 2) control sow diet with MOS nursery diet, 3) MOS sow diet with control nursery diet, and 4) MOS sow diet with MOS nursery diet. The average weaning weight of all piglets was 6.87 ± 1.04 kg. Each treatment was replicated with 7 pens of either 3 or 4 weaning pigs housed in a 1.2 m × 1.2 m raised wire floor pen equipped with a 4-hole, stainless steel feeder and a nipple waterer. Animals were allowed ad libitum access to feed and water.

The diets were based primarily on corn and soybean meal which were calculated to contain 3,404 kcal/g ME, 21.92% CP, and 1.38% lysine for the Phase 1 diet; 3,316 kcal/g ME, 20.80 % CP, and 1.21% lysine for the Phase 2 diet (Table 4.2). Minerals and vitamins were added to the diets to meet or exceed the NRC (1998) requirement estimate. No antibiotic was added to the diets.

Data and sample collection

Body weight and feed disappearance were recorded weekly through the fourth week. The experiment included Phase 1 (wk 0-2) and Phase 2 (wk 2–4). Blood samples were collected from each pig via the jugular vein using a V-trough on d 14.
and d 28 post-weaning. The blood samples were then centrifuged at 364 × g at 4 ºC for 20 min to obtain serum. Serum samples were stored at −20 ºC for potential immunoglobulin analysis.

Table 4.2. Percentage composition of the basal diet for nursery pigs (as-fed basis)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>D 0 – 14 (Phase 1) %</th>
<th>D 14 – 28 (Phase 2) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>49.20</td>
<td>59.86</td>
</tr>
<tr>
<td>Soybean meal, 48% CP</td>
<td>28.00</td>
<td>28.00</td>
</tr>
<tr>
<td>Dried whey</td>
<td>10.00</td>
<td>8.00</td>
</tr>
<tr>
<td>Spray dried plasma protein</td>
<td>1.50</td>
<td>0.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Grease</td>
<td>2.00</td>
<td>0.00</td>
</tr>
<tr>
<td>L-Lysine·HCl</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.90</td>
<td>0.75</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Salt</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Mineral mix¹</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Vitamin mix²</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Santoquin³</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Calculated nutrient composition

<table>
<thead>
<tr>
<th></th>
<th>ME, kcal/g</th>
<th>CP, %</th>
<th>Lysine, %</th>
<th>Calcium, %</th>
<th>Phosphorus, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3,404</td>
<td>21.92</td>
<td>1.38</td>
<td>0.81</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>3,316</td>
<td>20.80</td>
<td>1.21</td>
<td>0.71</td>
<td>0.62</td>
</tr>
</tbody>
</table>

¹ Provided (per kilogram of final diet): 160 mg Zn (ZnO); 128 mg Fe (FeSO₄·H₂O); 12.8 mg Cu (CuSO₄·5H₂O); 48 mg Mn (MnO); 1.6 mg I (CaI₂), 0.32 mg Se (Na₂SeO₃), 13.2 mg Ca (CaCO₃).

² Provided (per kilogram of final diet): 6,600 IU of vitamin A, 880 IU of vitamin D₃, 44 IU of vitamin E, 19.3 mg of vitamin K (as Menadione Sodium Bifulfite Complex), 8.8 mg of riboflavin, 22 mg of d-pantothenic acid, 44 mg of niacin, 33 μg of vitamin B₁₂, 220 μg of d-biotin, and 1.32 mg of folic acid.

³ Supplied 130 mg ethoxyquin per kg of basal diet.
4.2.3 Laboratory analysis

The blood samples were centrifuged at 364 × g at 4 °C for 20 min after to obtain serum. Colostrum and milk samples were centrifuged at 9,950 × g at 4 °C for 20 and 10 min, respectively, to separate fat from skim milk. After the fat layer was removed and discarded, the skimmed colostrum and milk samples were centrifuged at 39,800 × g at 4 °C for 45 and 20 min, respectively, to separate the whey fraction. Serum samples and whey fractions from colostrum and milk samples were stored at –20 °C before use for immunological analysis.

Total IgA, total IgG, and total IgM were measured in all serum and colostrum/milk whey samples by enzyme-linked immunosorbent assay (ELISA) test (pig IgA/IgG/IgM ELISA Quantitation Kit, Bethyl Laboratories Inc., Montgomery, TX) following the manufacturer's protocol. Detailed analysis procedure is described in Appendix III.

Approximately 10 mL of milk samples were stored as raw milk at – 20 °C before compositional analysis. The raw milk samples were thawed and diluted 4 fold with phosphorate buffered saline (PBS) before delivery to the milk laboratory of the Division of Regulatory Services, University of Kentucky to analyze milk component. The gross energy content of the complete milk was calculated from the concentration of protein, fat, and lactose, which contribute 16.4 kJ/g, 38.9 kJ/g, and 23.8 kJ/g respectively (Ramanau et al., 2004).

4.2.4 Statistical analysis

All data were analyzed by ANOVA in a completely randomized design with pen as the experimental unit. Analysis of variance was performed using the GLM
procedure of SAS (SAS Inst. Inc., Cary, NC). The model used for the sow experiment is:

\[ Y = k + t_i + e_i; \]

In this equation, the parameters represent:
- \( k \): a constant
- \( t_i \): the treatment effect
- \( e_i \): error term of the model

In addition, the model for evaluation of the 2 main effect (sow and nursery) and interaction is as follows:

\[ Y_{ij} = k + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij}; \]

In this equation, the parameters represent:
- \( k \): a constant
- \( \alpha_i \): the sow effect
- \( \beta_j \): the nursery effect
- \( (\alpha\beta)_{ij} \): the interaction of sow and nursery effect
- \( e_i \): error term of the model

The sow data were also analyzed using lactation length as covariate; nursery data were analyzed using initial BW as covariate.

4.3. **Results**

4.3.1 *Sow reproductive performances*

The effects of dietary MOS supplementation to sows are shown on Table 4.3. The length of gestation and lactation and feed intake during lactation was not affected by feeding MOS in the diet, but the sows fed MOS lost weight during lactation while sows fed the control diet gained a little (-7.69 vs. 0.54 kg, \( P < 0.01 \)).
The litter size did not differ between the MOS group and the control group; the litter weight at birth and at weaning for sows fed MOS also did not differ although it was more than 10% higher than sows fed the control diet (17.41 vs. 15.21 kg at birth; 63.75 vs. 55.17 kg at weaning, P > 0.10). When the weight is expressed as individual pig weight, then sows fed MOS had heavier piglets than control sows (total born; 1.61 vs. 1.45 kg, P < 0.10). However, the difference was significant only for the born-alive-piglets (1.65 vs. 1.47 kg, P < 0.05). After transferring piglets to balance the litter size within treatment, the piglets from sow fed MOS diet were still heavier at weaning (6.95 vs. 6.17 kg, P < 0.05). The distribution of individual pig weights is provided in Figure 4.1.

There was no difference between the control and MOS treatments on mortality at birth (i.e. percentage of stillborns) (9.85 vs. 9.95, P = 0.98), or at weaning (8.18 vs. 6.36 %, P = 0.63). Sow weight change during lactation and litter weight at weaning may be influenced by the lactation length. Although there is no significant difference of lactation length between treatments (18.92 vs. 18.36 d, P > 0.20), using lactation length as a covariate was conducted and, predictably, showed similar results (data presented in Table 4.4) that MOS treated sows still have heavier live piglets at birth (1.65 vs. 1.47 kg, P < 0.05) and at weaning (6.89 vs. 6.33 kg, P < 0.05).
Figure 4.1. Body weight distribution of pigs at various times.
Table 4.3. The effect of dietary MOS supplementation on reproductive performance in sows

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MOS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average parity</td>
<td>1.82</td>
<td>1.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length, d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestation&lt;sup&gt;1&lt;/sup&gt;</td>
<td>115.55</td>
<td>114.92</td>
<td>0.29</td>
<td>0.14</td>
</tr>
<tr>
<td>Lactation</td>
<td>18.36</td>
<td>18.92</td>
<td>0.50</td>
<td>0.43</td>
</tr>
<tr>
<td>Days to rebreed&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.33</td>
<td>4.75</td>
<td>0.18</td>
<td>0.12</td>
</tr>
<tr>
<td>Weight change, kg&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestation</td>
<td>32.02</td>
<td>30.52</td>
<td>4.78</td>
<td>0.82</td>
</tr>
<tr>
<td>D 101 - postfarrow</td>
<td>5.66</td>
<td>5.59</td>
<td>1.46</td>
<td>0.97</td>
</tr>
<tr>
<td>Lactation</td>
<td>0.54</td>
<td>-7.69</td>
<td>1.95</td>
<td>0.01</td>
</tr>
<tr>
<td>Lactation feed intake, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>95.75</td>
<td>100.91</td>
<td>5.18</td>
<td>0.56</td>
</tr>
<tr>
<td>ADFI</td>
<td>5.21</td>
<td>5.31</td>
<td>0.28</td>
<td>0.80</td>
</tr>
<tr>
<td>Litter size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10.45</td>
<td>10.92</td>
<td>0.99</td>
<td>0.74</td>
</tr>
<tr>
<td>Alive</td>
<td>9.36</td>
<td>9.85</td>
<td>0.96</td>
<td>0.72</td>
</tr>
<tr>
<td>Post-transfer&lt;sup&gt;4&lt;/sup&gt;</td>
<td>10.00</td>
<td>9.85</td>
<td>0.63</td>
<td>0.86</td>
</tr>
<tr>
<td>Weaning</td>
<td>9.00</td>
<td>9.15</td>
<td>0.48</td>
<td>0.82</td>
</tr>
<tr>
<td>Litter weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15.21</td>
<td>17.41</td>
<td>1.47</td>
<td>0.30</td>
</tr>
<tr>
<td>Alive</td>
<td>13.80</td>
<td>16.01</td>
<td>1.41</td>
<td>0.28</td>
</tr>
<tr>
<td>Post-transfer&lt;sup&gt;4&lt;/sup&gt;</td>
<td>15.20</td>
<td>16.08</td>
<td>1.04</td>
<td>0.55</td>
</tr>
<tr>
<td>Weaning</td>
<td>55.17</td>
<td>63.75</td>
<td>3.98</td>
<td>0.14</td>
</tr>
<tr>
<td>Average piglet weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.45</td>
<td>1.61</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Alive</td>
<td>1.47</td>
<td>1.65</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Post-transfer&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.52</td>
<td>1.64</td>
<td>0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>Weaning</td>
<td>6.17</td>
<td>6.95</td>
<td>0.24</td>
<td>0.03</td>
</tr>
<tr>
<td>Mortality, %&lt;sup&gt;5&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>9.85</td>
<td>9.95</td>
<td>3.61</td>
<td>0.98</td>
</tr>
<tr>
<td>Lactation</td>
<td>8.18</td>
<td>6.36</td>
<td>2.67</td>
<td>0.63</td>
</tr>
</tbody>
</table>

<sup>1</sup>Gestation length was calculated from the breeding to farrowing date.

<sup>2</sup>Control: n = 9; MOS: n = 12.

<sup>3</sup>Gestation wt. change: wt. difference between breeding and post-farrowing; D 101- postfarrow: wt. difference between the date before fed the experimental diet and post-farrowing; Lactation wt. change: weight difference between post-farrowing and weaning.

<sup>4</sup>No transferred piglet died before weaning. Pigs transferred to Control litters from non-experimental sows; pigs transferred within the MOS treatment only from sow in that treatment.

Table 4.4. The effect of dietary MOS supplementation on reproductive performance in sows with lactation length used as covariate for selected responses.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MOS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactation length, d(^1)</td>
<td>18.67</td>
<td>18.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days to rebreed(^2)</td>
<td>4.33</td>
<td>4.75</td>
<td>0.18</td>
<td>0.12</td>
</tr>
<tr>
<td>Weight change, kg(^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactation(^i)</td>
<td>0.34</td>
<td>-7.51</td>
<td>2.04</td>
<td></td>
</tr>
<tr>
<td>Lactation feed intake, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total(^1)</td>
<td>95.75</td>
<td>100.91</td>
<td>5.18</td>
<td>0.56</td>
</tr>
<tr>
<td>ADFI(^1)</td>
<td>5.21</td>
<td>5.31</td>
<td>0.28</td>
<td>0.80</td>
</tr>
<tr>
<td>Litter size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning</td>
<td>9.00</td>
<td>9.15</td>
<td>0.48</td>
<td>0.82</td>
</tr>
<tr>
<td>Litter weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning(^1)</td>
<td>55.52</td>
<td>62.82</td>
<td>3.93</td>
<td>0.20</td>
</tr>
<tr>
<td>Average piglet weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning(^1)</td>
<td>6.33</td>
<td>6.89</td>
<td>0.16</td>
<td>0.02</td>
</tr>
<tr>
<td>Mortality(^4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactation(^i)</td>
<td>8.12</td>
<td>6.38</td>
<td>2.85</td>
<td>0.67</td>
</tr>
</tbody>
</table>

\(^1\) Results are presented as LSMEANS with Lactation Length as covariate.
\(^2\) Control: n = 9; Celmanax: n = 12.
\(^3\) Gestation weight change is the weight difference between breeding and post-farrowing; D 101-postfarrow is the weight difference between the day before sow was fed the experimental diet and post-farrowing; Lactation weight change is the weight difference between post-farrowing and weaning.
Table 4.5 The effect of dietary MOS supplementation on sow milk composition

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MOS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Early lactation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td>8.22</td>
<td>8.99</td>
<td>0.55</td>
<td>0.33</td>
</tr>
<tr>
<td>Protein, %</td>
<td>5.56</td>
<td>5.73</td>
<td>0.21</td>
<td>0.58</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>5.84</td>
<td>5.54</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Energy, MJ/kg(^2)</td>
<td>5.48</td>
<td>5.77</td>
<td>0.24</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>Late lactation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td>7.67</td>
<td>8.28</td>
<td>0.35</td>
<td>0.22</td>
</tr>
<tr>
<td>Protein, %</td>
<td>4.82</td>
<td>4.92</td>
<td>0.17</td>
<td>0.67</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>5.90</td>
<td>5.85</td>
<td>0.14</td>
<td>0.79</td>
</tr>
<tr>
<td>Energy, MJ/kg(^2)</td>
<td>5.10</td>
<td>5.35</td>
<td>0.15</td>
<td>0.25</td>
</tr>
</tbody>
</table>

1Early lactation milk sample was obtained on d 4-6 of lactation; late lactation milk sample was obtained on d 15-17 of lactation.

2Gross energy content of the milk was calculated from the concentrations of protein, fat, and lactose. The energy concentrations used were: lactose, 16.4 kJ/g; fat, 38.9 kJ/g; protein, 23.8 kJ/g (Ramanau et al. 2004).

4.3.2 Sow milk and serum immunoglobulin level during lactation

Table 4.5 provides the protein, fat, and lactose content of the sow milk. There were no differences in these milk components between treatments, but fat and protein content were numerically higher in MOS treated sow milk than control sow milk in both early lactation (fat: 8.99 vs. 8.22 %; protein: 5.73 vs. 5.56 %, P > 0.20) and late lactation (fat: 8.28 vs. 7.67 %; protein: 4.92 vs. 4.82 %, P > 0.20). Lactose content was numerically lower at both time points. Because 2 components were numerically increased and 1 decreased, there was no difference in the computed energy content.

Colostrum, milk, and serum immunoglobulin level are presented in Table 4.6. MOS treatment increased IgG level 10% in colostrum compared to the control treatment, but the result was not significant (57.85 vs. 51.54 mg/mL, P > 0.20).
Colostrum IgA and IgM level and both early and late lactation milk IgA, IgG, IgM were also numerically higher in MOS treatments, but none of them were significant.

Sow serum immunoglobulin level had similar results as milk samples. Sows with dietary MOS supplementation had numerically higher IgM before farrowing (8.55 vs. 6.80 mg/mL, P < 0.20), higher IgA and IgG after farrowing (IgA: 1.33 vs. 0.68 mg/mL; IgG: 11.08 vs. 5.67 mg/mL, P < 0.20), and higher IgM during late lactation (5.21 vs. 3.80, P < 0.05). There was no difference on piglet serum immunoglobulin during late lactation between treatments.
Table 4.6. The effect of dietary MOS supplementation on milk and serum immunoglobulin level, mg/mL.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MOS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-farrow sow serum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>1.18</td>
<td>1.20</td>
<td>0.12</td>
<td>0.91</td>
</tr>
<tr>
<td>IgG</td>
<td>13.80</td>
<td>14.51</td>
<td>1.13</td>
<td>0.65</td>
</tr>
<tr>
<td>IgM</td>
<td>6.80</td>
<td>8.55</td>
<td>0.74</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Post-farrow sow serum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
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<td></td>
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<tr>
<td>IgA</td>
<td>0.69</td>
<td>1.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>5.67</td>
<td>11.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>6.83</td>
<td>4.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Colostrum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>11.22</td>
<td>12.31</td>
<td>1.59</td>
<td>0.63</td>
</tr>
<tr>
<td>IgG</td>
<td>51.54</td>
<td>57.85</td>
<td>5.93</td>
<td>0.46</td>
</tr>
<tr>
<td>IgM</td>
<td>3.70</td>
<td>3.77</td>
<td>0.45</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Early lactation milk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>4.80</td>
<td>5.54</td>
<td>0.53</td>
<td>0.33</td>
</tr>
<tr>
<td>IgG</td>
<td>0.60</td>
<td>0.72</td>
<td>0.12</td>
<td>0.47</td>
</tr>
<tr>
<td>IgM</td>
<td>1.90</td>
<td>2.30</td>
<td>0.25</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Late lactation milk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N</td>
<td>11</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>3.44</td>
<td>4.10</td>
<td>0.41</td>
<td>0.27</td>
</tr>
<tr>
<td>IgG</td>
<td>0.20</td>
<td>0.26</td>
<td>0.03</td>
<td>0.12</td>
</tr>
<tr>
<td>IgM</td>
<td>1.02</td>
<td>1.26</td>
<td>0.13</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Late lactation sow serum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>0.88</td>
<td>1.04</td>
<td>0.10</td>
<td>0.29</td>
</tr>
<tr>
<td>IgG</td>
<td>15.84</td>
<td>15.75</td>
<td>0.99</td>
<td>0.95</td>
</tr>
<tr>
<td>IgM</td>
<td>3.80</td>
<td>5.21</td>
<td>0.45</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Late lactation pooled piglets serum</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>0.17</td>
<td>0.16</td>
<td>0.04</td>
<td>0.82</td>
</tr>
<tr>
<td>IgG</td>
<td>8.30</td>
<td>7.89</td>
<td>0.77</td>
<td>0.71</td>
</tr>
<tr>
<td>IgM</td>
<td>0.43</td>
<td>0.47</td>
<td>0.04</td>
<td>0.49</td>
</tr>
</tbody>
</table>

1Pre-farrow serum sample was obtained on the D 111-113 of gestation; Post-farrow serum samples and colostrum samples were obtained within 24 h of farrowing; Early lactation milk sample was obtained on D 4-6 of lactation; Late lactation milk sample, sow serum sample and piglets serum samples were obtained on D 15-17 of lactation.

2 Control: n = 9; MOS: n = 12
Nursery growth performance

The growth performance during the nursery period of piglets from sows fed with or without MOS is presented in Table 4.7. Piglets from sows fed MOS were heavier than those from sows fed the control diet at weaning and at each weekly weight period of the entire nursery period (P < 0.01). The weekly ADG and ADG of each phase of piglets from sow fed MOS were numerically higher than piglets from control sows, however, only for the total experimental period was the ADG of piglets from MOS treated sows significantly higher than that of piglets from control sows (444 vs. 405 g, P < 0.05).

There was no significant difference in AFDI and feed:gain ratio among sow treatments in each week, each phase, nor entire experimental period, but pigs from MOS-fed sows had numerical increases in ADFI. The MOS supplementation of nursery feeds did not affect growth performance nor was there an interaction between sow and nursery feed supplementation.

The growth performance during the nursery period can be affected by the initial BW of pigs in the nursery, thus, Table 4.8 shows the results of growth with initial BW as the covariate. The body weight of piglets from MOS treated sows were still heavier at Week 1 (8.00 vs. 7.65 kg, P = 0.08). Correspondingly, higher ADG is observed at Week 1 (159.03 vs. 109.51 g, P = 0.08). The differences of body weight and ADG at the other time period as well as differences of ADFI at any time period are not detected when covariate analysis with initial weight is performed. However, pigs from MOS treated sows exhibit reduced feed:gain ratio at Week 1 (1.38 vs. 2.55, P = 0.10), at Week 3(1.49 vs. 1.61, P = 0.08), and for the entire nursery period (1.53 vs. 1.61, P = 0.06) after correction with initial BW as covariate.
MOS supplementation in starter feed had no effects on BW, ADG, ADFI as well as feed:gain ratio with initial BW as covariate. No interaction between MOS supplementation in sow diet and in nursery diet is detected.
Table 4.7. Effects of dietary MOS supplementation on growth performance of weaned pigs.

<table>
<thead>
<tr>
<th>Treatments&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Sow Control MOS</th>
<th>Sow Pig S × P</th>
<th>P-value</th>
<th>SEM Sow Pig S × P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>6.49&lt;sup&gt;b&lt;/sup&gt; 6.52&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.27&lt;sup&gt;a&lt;/sup&gt; 7.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26</td>
<td><strong>0.007</strong> 0.93 0.95</td>
</tr>
<tr>
<td>Week 1</td>
<td>7.39&lt;sup&gt;a&lt;/sup&gt; 7.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.31&lt;sup&gt;b&lt;/sup&gt; 8.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25</td>
<td><strong>0.001</strong> 0.83 1.00</td>
</tr>
<tr>
<td>Week 2</td>
<td>9.95&lt;sup&gt;ab&lt;/sup&gt; 9.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.99&lt;sup&gt;a&lt;/sup&gt; 11.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40</td>
<td><strong>0.005</strong> 0.79 0.66</td>
</tr>
<tr>
<td>Week 3</td>
<td>13.83&lt;sup&gt;ab&lt;/sup&gt; 13.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.86&lt;sup&gt;a&lt;/sup&gt; 15.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46</td>
<td><strong>0.004</strong> 0.64 0.32</td>
</tr>
<tr>
<td>Week 4</td>
<td>18.28&lt;sup&gt;ab&lt;/sup&gt; 17.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.60&lt;sup&gt;a&lt;/sup&gt; 19.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64</td>
<td><strong>0.007</strong> 0.61 0.40</td>
</tr>
<tr>
<td><strong>Average daily gain, ADG, g</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Week 1</td>
<td>130 117</td>
<td>149 141</td>
<td>24.3</td>
<td>0.38 0.67 0.91</td>
</tr>
<tr>
<td>Week 2</td>
<td>365&lt;sup&gt;xy&lt;/sup&gt; 33&lt;sup&gt;y&lt;/sup&gt;</td>
<td>383&lt;sup&gt;xy&lt;/sup&gt; 400&lt;sup&gt;x&lt;/sup&gt;</td>
<td>27.3</td>
<td>0.13 0.78 0.37</td>
</tr>
<tr>
<td>Week 3</td>
<td>555&lt;sup&gt;xy&lt;/sup&gt; 497&lt;sup&gt;y&lt;/sup&gt;</td>
<td>554&lt;sup&gt;xy&lt;/sup&gt; 580&lt;sup&gt;x&lt;/sup&gt;</td>
<td>31.3</td>
<td>0.20 0.62 0.18</td>
</tr>
<tr>
<td>Week 4</td>
<td>635 609</td>
<td>677 672</td>
<td>33.6</td>
<td>0.13 0.64 0.75</td>
</tr>
<tr>
<td>Phase 1</td>
<td>247 225</td>
<td>266 270</td>
<td>20.2</td>
<td>0.12 0.66 0.50</td>
</tr>
<tr>
<td>Phase 2</td>
<td>595&lt;sup&gt;xy&lt;/sup&gt; 553&lt;sup&gt;y&lt;/sup&gt;</td>
<td>615&lt;sup&gt;xy&lt;/sup&gt; 626&lt;sup&gt;x&lt;/sup&gt;</td>
<td>28.3</td>
<td>0.11 0.58 0.35</td>
</tr>
<tr>
<td>Total</td>
<td>421&lt;sup&gt;ab&lt;/sup&gt; 389&lt;sup&gt;b&lt;/sup&gt;</td>
<td>441&lt;sup&gt;ab&lt;/sup&gt; 448&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.7</td>
<td><strong>0.05</strong> 0.51 0.29</td>
</tr>
<tr>
<td><strong>Average daily feed intake, ADFI, g</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>212 201</td>
<td>228 229</td>
<td>18.6</td>
<td>0.24 0.80 0.75</td>
</tr>
<tr>
<td>Week 2</td>
<td>509 511</td>
<td>528 553</td>
<td>30.1</td>
<td>0.31 0.64 0.70</td>
</tr>
<tr>
<td>Week 3</td>
<td>838 798</td>
<td>855 871</td>
<td>37.3</td>
<td>0.24 0.74 0.45</td>
</tr>
<tr>
<td>Week 4</td>
<td>1,054 1,031</td>
<td>1,105 1,117</td>
<td>51.6</td>
<td>0.19 0.91 0.73</td>
</tr>
<tr>
<td>Phase 1</td>
<td>360 356</td>
<td>378 391</td>
<td>21.9</td>
<td>0.24 0.83 0.69</td>
</tr>
<tr>
<td>Phase 2</td>
<td>947 915</td>
<td>980 994</td>
<td>41.4</td>
<td>0.18 0.83 0.58</td>
</tr>
<tr>
<td>Total</td>
<td>653 635</td>
<td>679 693</td>
<td>27.9</td>
<td>0.15 0.93 0.57</td>
</tr>
<tr>
<td><strong>Feed/gain ratio</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>1.92 2.33</td>
<td>1.65 1.97</td>
<td>0.46</td>
<td>0.49 0.43 0.92</td>
</tr>
<tr>
<td>Week 2</td>
<td>1.40&lt;sup&gt;y&lt;/sup&gt; 1.55&lt;sup&gt;x&lt;/sup&gt;</td>
<td>1.43&lt;sup&gt;xy&lt;/sup&gt; 1.39&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.06</td>
<td>0.32 0.39 0.14</td>
</tr>
<tr>
<td>Week 3</td>
<td>1.52&lt;sup&gt;xy&lt;/sup&gt; 1.64&lt;sup&gt;x&lt;/sup&gt;</td>
<td>1.55&lt;sup&gt;x&lt;/sup&gt; 1.50&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.06</td>
<td>0.34 0.58 0.14</td>
</tr>
<tr>
<td>Week 4</td>
<td>1.66 1.70</td>
<td>1.64 1.66</td>
<td>0.03</td>
<td>0.29 0.31 0.81</td>
</tr>
<tr>
<td>Phase 1</td>
<td>1.48 1.60</td>
<td>1.44 1.48</td>
<td>0.07</td>
<td>0.24 0.24 0.51</td>
</tr>
<tr>
<td>Phase 2</td>
<td>1.59 1.67</td>
<td>1.60 1.59</td>
<td>0.04</td>
<td>0.32 0.38 0.22</td>
</tr>
<tr>
<td>Total</td>
<td>1.55&lt;sup&gt;y&lt;/sup&gt; 1.64&lt;sup&gt;x&lt;/sup&gt;</td>
<td>1.55&lt;sup&gt;y&lt;/sup&gt; 1.55&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.03</td>
<td>0.17 0.23 0.20</td>
</tr>
</tbody>
</table>

<sup>abc</sup> Means with the same letter are not significantly different (P < 0.05).

<sup>xyz</sup> Means with the same letter are not significantly different  (P < 0.10).

<sup>1</sup> Pigs form 7 litters of each sow treatments were allotted to 2 nursery treatment diets, including the Control diet and the diet with 0.2% MOS, with 3 or 4 pigs per pen and 7 pens per treatment.

<sup>2</sup> Phase 1 is Week 1 and Week 2 postweaning; Phase 2 is Week 3 and Week 4 postweaning.
Table 4.8. Effects of dietary MOS supplementation on growth performance of weaned pigs with initial BW as covariate.

<table>
<thead>
<tr>
<th>Treatments¹</th>
<th>Sow</th>
<th>Control</th>
<th>MOS</th>
<th>SEM</th>
<th>P-value</th>
<th>Pig</th>
<th>S x P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>MOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>6.89</td>
<td>6.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>7.69</td>
<td>7.61</td>
<td>8.03</td>
<td>7.97</td>
<td>0.17</td>
<td>0.07</td>
<td>0.67</td>
</tr>
<tr>
<td>Week 2</td>
<td>10.38</td>
<td>10.06</td>
<td>10.58</td>
<td>10.64</td>
<td>0.30</td>
<td>0.26</td>
<td>0.66</td>
</tr>
<tr>
<td>Week 3</td>
<td>14.22</td>
<td>13.50</td>
<td>14.49</td>
<td>14.74</td>
<td>0.41</td>
<td>0.11</td>
<td>0.56</td>
</tr>
<tr>
<td>Week 4</td>
<td>18.87</td>
<td>17.95</td>
<td>19.04</td>
<td>19.25</td>
<td>0.54</td>
<td>0.23</td>
<td>0.49</td>
</tr>
<tr>
<td>Average daily gain, ADG, g²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>115</td>
<td>104</td>
<td>163</td>
<td>155</td>
<td>23.9</td>
<td>0.07</td>
<td>0.67</td>
</tr>
<tr>
<td>Week 2</td>
<td>384</td>
<td>350</td>
<td>364</td>
<td>381</td>
<td>26.0</td>
<td>0.85</td>
<td>0.73</td>
</tr>
<tr>
<td>Week 3</td>
<td>549</td>
<td>491</td>
<td>559</td>
<td>586</td>
<td>33.1</td>
<td>0.16</td>
<td>0.63</td>
</tr>
<tr>
<td>Week 4</td>
<td>665</td>
<td>635</td>
<td>649</td>
<td>644</td>
<td>29.8</td>
<td>0.92</td>
<td>0.55</td>
</tr>
<tr>
<td>Phase 1</td>
<td>250</td>
<td>227</td>
<td>264</td>
<td>268</td>
<td>21.5</td>
<td>0.26</td>
<td>0.66</td>
</tr>
<tr>
<td>Phase 2</td>
<td>607</td>
<td>563</td>
<td>604</td>
<td>615</td>
<td>29.1</td>
<td>0.45</td>
<td>0.56</td>
</tr>
<tr>
<td>Total</td>
<td>428</td>
<td>395</td>
<td>434</td>
<td>442</td>
<td>19.4</td>
<td>0.24</td>
<td>0.50</td>
</tr>
<tr>
<td>Average daily feed intake, ADFI, g²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>205</td>
<td>195</td>
<td>234</td>
<td>236</td>
<td>19.4</td>
<td>0.12</td>
<td>0.81</td>
</tr>
<tr>
<td>Week 2</td>
<td>529</td>
<td>529</td>
<td>509</td>
<td>534</td>
<td>28.9</td>
<td>0.81</td>
<td>0.65</td>
</tr>
<tr>
<td>Week 3</td>
<td>848</td>
<td>807</td>
<td>846</td>
<td>862</td>
<td>39.3</td>
<td>0.55</td>
<td>0.74</td>
</tr>
<tr>
<td>Week 4</td>
<td>1099</td>
<td>1,071</td>
<td>1,063</td>
<td>1,075</td>
<td>45.8</td>
<td>0.75</td>
<td>0.85</td>
</tr>
<tr>
<td>Phase 1</td>
<td>367</td>
<td>362</td>
<td>372</td>
<td>385</td>
<td>22.9</td>
<td>0.60</td>
<td>0.84</td>
</tr>
<tr>
<td>Phase 2</td>
<td>974</td>
<td>939</td>
<td>954</td>
<td>968</td>
<td>40.1</td>
<td>0.91</td>
<td>0.78</td>
</tr>
<tr>
<td>Total</td>
<td>670</td>
<td>651</td>
<td>663</td>
<td>676</td>
<td>27.4</td>
<td>0.76</td>
<td>0.90</td>
</tr>
<tr>
<td>Feed/gain ratio²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>2.36</td>
<td>2.73</td>
<td>1.23</td>
<td>1.54</td>
<td>0.38</td>
<td>0.01</td>
<td>0.35</td>
</tr>
<tr>
<td>Week 2</td>
<td>1.37</td>
<td>1.53</td>
<td>1.46</td>
<td>1.42</td>
<td>0.07</td>
<td>0.91</td>
<td>0.36</td>
</tr>
<tr>
<td>Week 3</td>
<td>1.55</td>
<td>1.67</td>
<td>1.52</td>
<td>1.47</td>
<td>0.06</td>
<td>0.07</td>
<td>0.58</td>
</tr>
<tr>
<td>Week 4</td>
<td>1.66</td>
<td>1.69</td>
<td>1.64</td>
<td>1.67</td>
<td>0.03</td>
<td>0.55</td>
<td>0.32</td>
</tr>
<tr>
<td>Phase 1</td>
<td>1.49</td>
<td>1.62</td>
<td>1.42</td>
<td>1.46</td>
<td>0.07</td>
<td>0.16</td>
<td>0.25</td>
</tr>
<tr>
<td>Phase 2</td>
<td>1.60</td>
<td>1.68</td>
<td>1.59</td>
<td>1.57</td>
<td>0.04</td>
<td>0.14</td>
<td>0.38</td>
</tr>
<tr>
<td>Total</td>
<td>1.57</td>
<td>1.65</td>
<td>1.53</td>
<td>1.53</td>
<td>0.03</td>
<td>0.06</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different according to LSMEAN with covariate as initial weight (P < 0.05).

Means with the same letter are not significantly different according to LSMEAN with covariate as initial weight (P < 0.10).

¹ Pigs form 7 litters of each sow treatments were allotted to 2 nursery treatment diets, including the Control diet and the diet with 0.2% MOS, with 3 or 4 pigs per pen and 7 pens per treatment.

² Phase 1 is Week 1 and Week 2 postweaning; Phase 2 is Week 3 and Week 4 postweaning.
4.4. Discussion

Sow research is traditionally hampered by inadequate numbers of observations to detect statistically significant differences between treatments. And, when low sow numbers are used, even when a study demonstrates significance in 1 response measure, another measure may not be affected statistically even when it is well accepted to be biologically related to the one that was affected. While statistical significance is necessary to ascribe true treatment effects, evaluation of consistent biological movement in responses, of large percentage changes in responses, and of patterns of responses reported in the literature may provide inferences relative to how to interpret the results. The current study with less than 15 sows per treatment is a study with low sow numbers.

*MOS effects at lactation*

Feeding during pregnancy is important because it determines the litter size and subsequent viability of piglets at birth, the amount of mammary tissue the sow contains at parturition as well as the potential milk production of the sow during lactation (Pluske et al., 1995). There is no direct evidence that MOS can increase the nutrient absorption, but there is much indirect evidence to show that MOS has the potential to increase reproductive performances. For instance, Maxwell et al. (2003) reported that dietary MOS supplementation may decrease the days back to estrus (6.4 vs. 5.9 d; $P \geq 0.10$); O’Quinn et al. (2001) and Funderburg (2002) showed that pre-weaning piglet mortality was reduced in MOS-fed sows.

In the current study, MOS supplementation in the diet for sows in late gestation (14 d prior to farrowing) and throughout lactation has no effect on the
number of pigs born alive and weaned as well as preweaning mortality, which is consistent with Newman and Newman (2001), O’Quinn et al. (2001), Medal et al. (2004), and Maxwell et al. (2003). When dietary treatments are assigned about 2 weeks before farrowing, there would not be an expected diet effect. Thus equal litter size is more of an indication of an appropriate manner of allotment of sows.

Hall et al. (1987) observed that the survival rate during suckling of pigs that weigh less than 0.5 kg was only 7.7% while the survival rate of pigs weighing over 1.7 kg was over 95%. In the current study, the average piglet weight from MOS-fed sow is 1.65 kg and their preweaned mortality is 6.38%, which is close to the number that Hall et al. (1987) reported. The piglet weight from the control group is significantly lighter than the piglets from MOS group (1.65 vs. 1.47 kg; P = 0.04). The survival rate of pig at this weight reported in Hall et al. (1987) is approximately 85%, which is smaller than the result in the present study. The undetected difference of preweaned mortality between MOS group and the control group may be due to the clean and low-stress environment.

In addition, the piglet weight is also improved at weaning (6.95 vs. 6.17; P = 0.03), which is consistent with Medal et al. (2004), and Maxwell et al. (2003). The improved growth performance of piglets during suckling may result from the content of the milk. Fat and lactose content supply the energy for the neonatal pigs while protein content provides the immune protection and the essential amino acids. In the present study, the fat and protein levels in the milk of MOS-fed sows are numerically higher than the milk from control sows (P > 0.20). The gross energy content in the MOS-fed sow milk (calculated by the equation from Ramanau et al., 2004) is also increased 5% compared with the control sow milk (P > 0.25).

Meanwhile, IgA, IgG, and IgM concentrations in the colostrum are also numerically increased. Newman and Newman (2001) reported that colostral IgG and
IgM from MOS-fed sows are higher than that in control sows (IgG: 42.15 vs 35.65 mg/mL, P = 0.16; IgM: 4.40 vs. 3.16, P = 0.04). Similarly, O'Quinn et al. (2001) showed that IgG concentrations in colostrum respond significantly to MOS supplementation (58.53 vs 48.42 mg/mL, P = 0.007), as well as IgM (2.73 vs. 2.41 mg/mL, P = 0.03), and IgA (11.78 vs. 10.97 mg/mL, P = 0.06). Furthermore, the immunoglobulin concentration is increased at least 15% in the early (d 4-6 of lactation) and late (d 15-17 of lactation) milk from MOS-fed sow compared with the milk from the control sows, though the differences are not significant (P = 0.12-0.48).

Increasing nutrient intake during the last 30 days of gestation may aid the fetal growth since the fetus grows rapidly during this period (Pluske et al., 1995). In addition, it has been determined that the heavier piglet at birth is also heavier at weaning (Smith et al., 2007; Tokach et al., 1992,). The reason for having heavier piglets at birth from sows with MOS supplementation is not fully understood, but the result of heavier piglets at weaning is probably due to the increased weight at birth and the potentially improved milk quality.

**MOS effects at nursery**

There are many reports showing the benefits of dietary MOS supplements on growth performance of weaning pigs. Miguel et al. (2002) reviewed 49 papers and concluded that there are improvements in weight gain and feed conversion ratio when MOS is introduced into the weanling pig diet. However, there is not much study on the effect of MOS supplementation in sow diets their weaned pigs.

In the present study, weanling pigs from MOS-fed sows were significantly heavier at weaning (the beginning of the nursery period) and during the entire nursery period (P < 0.01). Though there is no difference in the weekly reports of the pigs’
daily gain between pigs from MOS-fed sows or control sows, the overall, cumulative, daily weight gain is improved by MOS supplementation to sow diets (444 vs. 404 g/d, P < 0.05). This result is similar to Gracia et al. (2004) who reported that weaned pigs, which are coming from sows fed with MOS during their lactation periods, grew more than piglets from control sows independently from their dietary treatment after weaning (339 vs. 363 g/d, P < 0.05).

Adding MOS into the nursery diet has no effect on the weaned pigs’ body weight and their weight gain. Moreover, the feed intake and feed:gain ratio is not affected by MOS supplementation in sow diet or nursery diet. However, Gracia et al. (2004) reported that piglets supplemented with MOS tended to have improved feed conversion rates than control piglets (1.50 vs. 1.39, P = 0.06). No interactions between supplying MOS in sow diet and nursery diet were detected in the current study and the study from Gracia et al. (2004).

Wolter and Ellis (2001) reported that pigs’ weights during nursery and growing are affected by their weaning weights. Thus, the results of growth were adjusted with initial BW as the covariate. After adjusting the effects by the initial weight, the improvement of body weight and weight gain is not profound as before correction. However, the difference of feed:gain ratio is improved by sow treatment at Week 1 (1.38 vs. 2.55, P = 0.01), Week 3 (1.49 vs. 1.61, P = 0.08) and overall period (1.53 vs. 1.61, P = 0.06). This result may indicate that MOS supplementation in sow diet affects the growth performance during nursery. MOS supplementation during nursery has no effects on growth performance, which is consistent with the previous work (Chapter 3).
4.5. Implication

The present study shows that MOS supplementation in sow diet increases the piglet weight at birth, at weaning and throughout the nursery period. This result suggests that adding MOS to sow diets may increase the profit of the swine producer due to the rapid growth rate of the piglets. Meanwhile, the milk composition and immunoglobulin concentration from the MOS-fed sow are numerically increased, which indicates that the immune system may be improved by MOS supplementation. However, further studies need to be conducted to evaluate the mechanism of MOS influence on this parameter.
Abstract

The effect of MOS on sow reproductive performance and piglet growth performance was evaluated in this study. A total of 28 sows with an average parity of 1.63 ± 0.92 were assigned to 2 dietary treatments, including 1) control diet and 2) the control diet with MOS product. The dietary treatments were started 14 days before the expected farrowing date, or approximately day 102 of gestation, and continued throughout the lactation period. After weaning, a total of 104 piglets were allotted within litter to a 2 × 2 factorial arrangement with the experimental treatments as: 1) control sow diet with control nursery diet, 2) control sow diet with MOS nursery diet, 3) MOS sow diet with control nursery diet, and 4) MOS sow diet with MOS nursery diet. There were no significant differences in litter size and litter weight between control sows and MOS-fed sows. However, the average piglet weight from MOS treated sows was heavier at birth and weaning compared with control sows (1.65 vs. 1.47 kg, P = 0.04 and 6.95 vs. 6.17 kg, P = 0.03, respectively). The milk composition was also not affected by the treatment. Immunoglobulin levels of colostrum and milk were numerically greater in sows receiving MOS than control sows (colostrum: IgG 57.9 vs. 51.5 mg/mL; IgA 12.3 vs. 11.2 mg/mL; IgM 3.8 vs. 3.7 mg/mL; early lactation milk: 0.7 vs. 0.6 mg/mL; IgA 5.5 vs. 4.8 mg/mL; IgM 2.3 vs. 1.9 mg/mL; late lactation milk: 0.3 vs. 0.2 mg/mL; IgA 4.1 vs. 3.4 mg/mL; IgM 1.3 vs. 1.0 mg/mL) but differences were not statistically significant. Piglets from MOS treated sows were heavier throughout the nursery period (Wk 0 7.3 vs. 6.5 kg, P = 0.007; Wk 2 11.0 vs. 9.8 kg, P = 0.005; Wk 4 19.7 vs. 17.8 kg, P = 0.007), but the effect on growth rate and feed intake were not significant. MOS supplementation during the nursery period had no effect on growth performance. In conclusion, adding the MOS product to sow diets
during the end of gestation throughout lactation increases both piglet birth and weaning weight, as well as the subsequent growth rate during the nursery.
Chapter 5. Summary

Antibiotics have been used for improving the animals’ growth performance and health (Cromwell, 2001). However, due to health concerns of the consumers on animal products and environment concerns, alternative products need to be provided to replace the usage of the antibiotics. Mannans oligosaccharide (MOS) has been reported to have an effect on enhancing the growth performance and/or immunocompetence in various species (Pettigrew, 2000; Parks et al., 2001; Sim et al., 2004; Mourão et al., 2006; Hooge, 2006). The present study confirms that there are benefits from a MOS product (some benefit in a disease challenge as modeled in LPS challenge and benefit for sows). However, this study did not demonstrate some of the reported benefits that are observed in published literature (e.g., increased growth in weanling pigs).

The mechanism of MOS improving the performances is purportedly from blocking the adherence of the microorganisms to the intestinal wall, thus preventing pathogens from colonizing and proliferating in the gut (Newman, 1994; Kelly, 2004). Pluske et al. (1997) stated that pathogens can only cause disease when they colonize and infect the epithelial cells of the intestine. As a result, reduced pathogen colonization indicates a healthy gastrointestinal tract and enhanced digestibility, which consequently leads to improved growth performance of the animals. Thus, improved gut health in sows may indicate that improved nutrient absorption or availability may have contributed enhanced reproductive performances in the sows.

Supplying dietary MOS in weaning pigs improve their growth rate, feed intake, and feed conversion rate (Miguel et al., 2002). However, this effect is not always consistent. Castillo et al. (2008) also reported that the improvement of growth...
performance was only significant at early phase of nursery, which is consistent with the current study (Chapter 3).

In the current study, LPS was administrated to evaluate the immune responses of weaning pig with dietary MOS supplementation. LPS is the endotoxin extracted from the outer membrane of gram-negative bacteria which trigger strong immune responses in animals, including reduced food intake, inactivity, and fever (Johnson and von Borell, 1994; Warren et al., 1997). Dietary MOS supplementation did not affect the weight gain, feed intake as well as serum cortisol and TNF-α concentration after LPS challenge, compared with control diet-fed, LPS-injected group. However, supplemental MOS did alleviate the LPS challenge responses on respiratory rate and rectal temperature, which suggested that MOS may provide some benefits for weaning pigs during the immune challenge.

MOS is beneficial not only for the growth performance in weanling pigs, but also for the reproduction in sows. In the current study (Chapter 4), MOS supplementation in sow diet during late gestation (14 d prior to farrowing) and throughout lactation had no effect on the number of pig born alive and weaned as well as preweaning mortality. However, the piglet weight is improved at birth and at weaning, which are consistent with Medal et al. (2004), and Maxwell et al. (2003).

The improved growth performance of piglets during suckling period may result from the contents of the milk. In the present study, the fat and protein levels as well as the gross energy in the milk of MOS-fed sows are numerically higher than the milk from control sows (P > 0.20). Additionally, IgA, IgG, and IgM concentrations are also numerically higher in the colostrum from sows with supplemental MOS compared to control sows. Similar results are observed in Newman and Newman (2001) and O’Quinn et al. (2001). Immunoglobulin concentration is also increased in
the early (d 4-6 of lactation) and late (d 15-17 of lactation) milk from MOS-fed sow compared with the milk from the control sows. Although the differences are not significant (P = 0.12-0.48), the 15% improvement may imply that the positive effect of MOS supplementation could be verified rather easily if there is a larger sample size.

The subsequent experiment of supplying MOS into the diets of nursery pigs (Chapter 4) showed that heavier weaned pigs from MOS-fed sows are significantly heavier at the entire nursery period (P < 0.01), so they maintain their weight advantage and actually may magnify it. Gracia et al. (2004) reported similar results that weaned pigs, which are coming from sows with supplemental MOS in the diet, grew faster than piglets from control sows. However, adding MOS into the nursery diet in this study again had no effect on the body weight and weight gain. Because there were no interactions between supplying MOS in sow diet and nursery diet, there does not appear to be any particular benefit, or need, of using the MOS product in both diets.

It has been demonstrated that a heavier pig at birth tends to be heavier at weaning (Smith et al., 2007; Hall et al., 1987). Similarly, a high weaning weight usually implies rapid and healthy growth after weaning and all the way through to slaughter (Wolter and Ellis, 2001; Tokach et al., 1992). Moreover, the preweaning survival rate of a heavier piglet is also higher than that of a lighter piglet (Smith et al., 2007; Hall et al., 1987). These elevated growth performance and survival rate observations indicate an increased profit for swine producers with more pigs for market, less turnover rate for facilities, less usage of potential medical or nutrient supplements cost for treating weaker pigs with heavier birth weights.
Pettigrew et al. (2005) calculated the economic effect of feeding Bio-MOS (1 of the MOS products) to sows. The increased survival rate (2.1%) with 10.2 pigs born alive per litter resulted in the extra profit of $6.30 for a litter (assuming a weaned piglet has a value of $30). Also, sows that received MOS had fewer days between post-weaning and next estrus (1.27 d) in their example, which resulted in an extra $0.61 saved (assuming the cost of an unproductive sow is $0.48/d). In addition, the increased weaning weight (0.2 kg) with 9.1 pigs weaned/litter contributed extra profit of $1.82 for a litter (assuming the additional weight of a pig is worth $1.00/kg). The increased growth rate during the nursery period (24 g/d in 32 d of nursery) with 9.1 pigs weaned/litter contributed extra profit of $6.99 for a litter. However, heavier pigs had increased feed intake (31 g/d in a 32-d nursery period) which was an extra cost of $1.81 for the feed (assuming the feed price is $0.2/kg).

Assuming that the cost of a MOS product is $3.50/kg and the MOS intakes in gestation (21 d) and in lactation (21 d) are 4 g and 6 g, respectively, then the cost of MOS per sow/litter would be $0.74. After adding up these extra profits and costs, the MOS effect resulted in an estimated net benefit of approximately $13 per litter.

\[
\begin{align*}
10.2 \text{ pigs born alive/litter} \times 2.1\% &= 0.21 \text{ pigs saved/litter} \\
0.21 \text{ pigs/litter} \times $30/\text{weaned pig} &= $6.30 \text{ extra profit/litter} \\
1.27 \text{ d shorter} \times $0.48/\text{d of an unproductive sow} &= $0.61 \text{ saved/litter} \\
0.2 \text{ kg/pig} \times $1.00/\text{kg} \times 9.1 \text{ pigs weaned/litter} &= $1.82 \text{ extra profit/litter} \\
24 \text{ g/pig/d} \times 32 \text{ d} \times 9.1 \text{ pigs weaned/litter} \times $1.00/\text{kg} &= $6.99 \text{ extra profit/litter} \\
31 \text{ g/pig/d} \times 32 \text{ d} \times 9.1 \text{ pigs weaned/litter} \times $0.20/\text{kg} &= $1.81 \text{ extra cost/litter}
\end{align*}
\]
$3.5/kg of MOS × (4 g/d × 21 d gestation + 6 g/d × 21 d lactation)

= $0.74 extra cost/litter

$ 6.30 + $0.61 + $1.82 + $6.99 - $1.81 – $0.74

= $13.17 net profit/litter.

Applying the research results presented in Chapter 4 to the equation developed by Pettigrew et al. (2005) showed approximately $19 of net profit per litter when MOS was introduced into the sows’ diet.

9.36 pigs born alive/litter × 1.82% = 0.17 pigs saved/litter

0.17 pigs/litter × $30/weaned pig = $5.10 extra profit/litter

0.36 d longer × $0.48/d of an unproductive sow = $0.17 extra cost/litter

0.78 kg/pig × $1.00/kg × 9.0 pigs weaned/litter

= $7.02 extra profit/litter

40 g/pig/d × 28 d × 9.0 pigs weaned/litter × $1.00/kg

= $10.08 extra profit/litter

40 g/pig/d × 28 d × 9.0 pigs weaned/litter × $0.20/kg

= $2.02 extra cost/litter

$3.5/kg of MOS × (3.6 g/d × 14 d gestation + 12 g/d × 19 d lactation) = $0.98 extra cost/litter

$ 5.10 - $0.17 + $7.02 + $10.08 - $2.02 – $0.98

= $19.03 net profit/litter.

Overall, dietary MOS supplementation in the nursery diet may have no effects on the weaning pigs’ growth performance (at least with the product evaluated in this research), but may have some beneficial influence on pigs under immune challenge. In addition, introducing MOS to the sow diet during late gestation and throughout
lactation can potentially improve the reproductive characteristics, i.e. litter size, litter weight, and mortality, milk composition, as well as the neonatal piglets’ growth during suckling and nursery. These improvements imply that MOS supplementation may be potentially used as the alternative product for antibiotic and lead to increased profits for a swine producer.
Appendix I

Assay to determinate serum cortisol concentration

The Coat-A-Count Cortisol kit (TKCO1, Diagnostic Products Corporation, Los Angeles, CA) was to measure the cortisol content. The shelf life of the cortisol kit was 60 days maximum, because the half-life of the $^{125}$I was 60 days. The kit should be ordered a few days before the use of the kit because of the short shelf life.

Samples and standards:

The serum samples from individual pigs were measured in duplicate at different time points (0, 2, 4, 8, 12, and 24 h post-injection). The kit supplied 1) the cortisol Ab-coated tubes, which coated with antibodies to cortisol, 2) $^{125}$I cortisol solution with blocking agents for steroid-binding protein, and 3) cortisol calibrators used as standards. Five standards (1.0, 5.0, 10.0, 20.0, and 50.0 μg/dL) and a blank were provided by the kit and measured in triplicate. In addition, label 6 plain (uncoated) polypropylene tubes (Fisher-Scientific, Pittsburg, PA) for total counts (TC) and non-specific binding (NSB) in triplicates. All components were used at room temperature.

Assay procedures:

1) A 25- μL sample of the 0 calibrator (blank), provided by the kit, was pipetted into the bottom of the non-specific binding and blank tubes. All standard calibrators supplied by the kit and samples were pipetted in the amount of 25 μL into the bottom of their assigned tubes.
2) After all standards, blanks, and samples were added to the tubes, 1.0 ml of $^{125}$I cortisol was added to all tubes and the total count tubes within 10 min, and then vortex to ensure the solution mixed evenly.

3) All tubes, except the total count tubes, were incubated in a 37°C water bath for 45 min.

4) Following the water bath incubation, decanted all tubes, except the total count tubes, thoroughly by turning the tubes up-side down allowing them to drain for 2 or 3 minutes on absorbance cloth to remove residual moisture.

5) The tubes were then read for 1 minute using a gamma counter (Cobra Auto-Gamma 5000 series, Gamma Counting Systems with CobraCon Data File Conversion Software; Packard Instrument Co., Meriden, CT).

**Result calculation:**

To obtain results in terms of concentration of the calibration curve, first calculate for the average percentage of binding (%B).

\[
\% \ B = \frac{\text{count of sample/standard} - \text{NSB count}}{\text{maximum binding (blank) count}} \times 100
\]

The standard curve was plotted with the %B and the concentration of each standard solution in different level. The gamma counter software fitted the standard curve using fourparameter logistics and calculated the concentration of the cortisol in the samples using the fitted standard curve.
Appendix II

Validation of assay to measure serum TNF-α concentration

The TNF-α kit (EP2TNFA; Pig TNF-α Colorimetric ELISA Kit; Pierce Endogen, Rockford, IL) was used to analyze TNF-α concentrations in the porcine serum. The validation of the kit and the validation of the 2 h post-injection time as appropriate to measure the serum TNF-α concentration in relation to the LPS injection (50 μg/kg BW) was determined using serum sampled from pigs in the preliminary experiment and the resulted suggested by Webel et al. (1997).

Samples used:

The blood samples were collected before and at 2 hr after injection via the jugular vein from pigs using a V-trough. The blood samples were then centrifuged at 1390 x g at 4 ºC for 20 min to obtain serum. These samples were stored at -20 ºC for future analysis.

Reagent preparation:

Sample diluent and wash buffer are prepared the day before the analysis by recombining the package of each provided in the kit with nanopure distilled water to the volume introduced on the package. The sample diluent is stored in the refrigerator while the wash buffer is stored in the room temperature.

Eight microcentrifuge tubes was labeled 2000, 1000, 500, 250, 125, 62.5, 31.3, and 0 pg/ml. The standards were prepared on the day of the analysis with recombinant porcine TNF-α provided by the kit. Reconstitute lyophilized standard in
nanopure, distilled water, and the recombinant TNF-α was brought to volume as indicated on the vial. Pipette 200 μL of sample diluent into each prelabeled microcentrifuge tube. A 200-μL standard solution, the reconstituted recombinant TNF-α was pipetted into the 2000 pg/mL tube. The tube was mixed by gently flushing the solution in the pipette tip. A 200-μL sample of the 2000 pg/ml TNF-α solution was then pipetted into the 1000 pg/ml tube and mixed gently. The serious dilution by transferring the previously prepared solution to the next tube was repeated to create all standards. The blank, marked 0 pg/mL, contained only sample diluent.

The samples were thawed at room temperature on the day of analysis and were diluted to 4-fold by adding 50 μL sample to 150 TNF-α sample diluent. No sample was thawed and used twice. All standards and blank were measured in duplicate while the samples were measured in triplicate. The ELISA plate map was determined and labeled prior to analysis (Figure A.2). During the assay, all components were used at room temperature.

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Figure A2-1. A ELISA plate map used for the analysis of TNF-α. The standards occupied column 1 and 5.
Assay procedures:

1) Add 50 μL of sample diluent, provided by the kit, to each well on a plate.
2) After the sample diluent was added to each well, 50 μL of the standards and samples were added to the respective plate wells as marked on the map.
3) The plate was covered and incubated for 2 h at room temperature (20-25°C) using an adhesive plate cover strip provided by the kit.
4) After the 2 h incubation, the plate was washed 3 times using the wash solution provided by the kit with the automated wash machine (EL404, Micorplate autowasher, BIO-TEK Instruments, Inc., Winoski, VT) and then pat onto paper towel to remove residual moisture.
5) Using a multi-tip pipette, 100 μL of biotinylated antibody reagent provided by the kit was pipetted to each well. The plate was then covered with an adhesive cover strip and allowed to incubate for 1 h at room temperature.
6) After the incubation period, the plate was again washed 3 times and decanted as mentioned previously.
7) Using the multi-tip pipette, 100 μL of streptavidin-HRP reagent provided by the kit was pipetted to each well. The plate was again covered using an adhesive cover strip and allowed to incubate for 30 min at room temperature.
8) The plate was again washed 3 times and decanted as mentioned previously.
9) Using the multi-tip pipette, 100 μL of TMB substrate solution provided by the kit was pipetted to each well.
10) The plate was not covered and placed in a location that it was not in direct light and allowed to incubate for 30 min at room temperature.

11) After incubation, 100 μL of stop solution provided by the kit was pipetted to each well using a multi-tip pipette. The plate was not washed or decanted after incubation.

12) The plate was then read on an ELISA plate reader (VERSAmax Microplate Reader, MDS Analytical Technologies, Inc., Sunnyvale, CA) located in the Agronomy Laboratory, Department of Plant and Soil Science, University of Kentucky to measure the absorbance at a wavelength of 450nm minus 550nm. The plate were read within 30 min after the stop solution was added to the wells.

*Calculation of results:*

The plate was read at the wavelength of 450um and 550um. The standard curve is generated by plotting the average absorbance (450nm minus 550nm) obtained for each of the standards in the vertical (Y) axis vs. the corresponding pig TNF-α concentrations on the horizontal (X) axis. The standard curve is showed in Figure A2-2. The amount of pig TNF-α in each sample is determined by interpolating from the sample absorbance value using the standard curve and multiply to 4 (the dilution rate that sample was used).
$y = 492.07x^2 + 605.4x - 12.461$

$R^2 = 0.9998$

Figure A2-2. A standard curve used to derive the unknown serum TNF-$\alpha$ concentration.
Appendix III

Assay to determine the immunoglobulin content in pig milk and serum

The immunoglobulin ELISA kits (pig IgA [E100-102]; IgG [E100-104]; IgM [E100-100] ELISA Quantitation Kit, Bethyl Laboratories Inc., Montgomery, TX) is quantitatively measuring the immunoglobulin content in biological samples, i.e. serum, plasma, milk). The shelf life of the kit is a year stored at 2-8 ºC. The procedure and reagent preparation are following the manufacturer's protocol.

Samples used:

Blood samples from the sows and the piglets were collected by jugular venipuncture. The blood samples were centrifuge at 364 × g at 4 ºC for 20 min to obtain serum. The serum samples of the piglets used for analysis were pooled from 5 piglets in the mid-weight range of each litter in same ratio (approximately 0.1 mL from each piglet); the remaining serum was kept individually as backup.

Colostrum or milk was hand-expressed from third and forth functional teat of both sides for a total collection of approximately 50 mL. A 1 mL oxytocin (20 USP) was administered intramuscular around neck and shoulder area to facilitate collection of the colostrums and milk samples. Colostrum and milk samples were centrifuged at 9,950 × g at 4 ºC for 20 and 10 min, respectively, to separate fat from skim milk. After the fat layer was removed and discarded, the skimmed colostrum and milk samples were centrifuged at 39,800 × g at 4 ºC for 45 and 20 min, respectively, to separate the whey fraction. Serum samples and whey fractions from colostrum and milk samples were stored at –20ºC before use for immunological analysis.
Reagent preparation:

Wash buffer, sample diluent, blocking solution, and coating buffer are prepared by recombining the package with nanopure distilled water to the volume introduced on the package. The stop solution (2M H$_2$SO$_4$) was prepared by diluting from high concentrated (95%) sulfate solution. Coating buffer, wash buffer, and stop solution are store in room temperature while the blocking solution and sample diluent are store at 2-8 °C before analysis. All components were used at room temperature.

Samples, standards, and HRP conjugate solution were diluted with the sample diluents on the day of analysis. The standards were prepared in the concentration of 0, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 ng/mL for examining IgA and IgM; 0, 7.8, 15.6, 31.3, 62.5, 125, 250, and 500 ng/mL for examining IgG. The serious diluted procedure is similar to the previous description in Appendix II. The HRP solution also diluted to 1:75,000 (IgA and IgG) or 1: 50,000 (IgM) suggested by the manufactory’s protocol. Capture antibody solution was prepared by adding 0.1 mL antibody solution provided in the kit to 9.9 mL coating solution on the day of analysis. The enzyme substrate solution was prepared by mixing the 2 substrate solution provided in the kit at the same ratio. The enzyme solution need to be fresh prepared before using for analysis.

The samples were thawed at room temperature on the day of analysis and were diluted with sample diluent. The dilution rates of the samples were estimated by the absorption value of the samples of the preliminary experiment. No sample was thawed and used twice. All standards and blank were measured in triplicate while the samples were measured in 2 dilutions and triplicate for each dilution. The ELISA plate map was determined and labeled prior to analysis (Figure A.3-1). All the components and the examination were conducted in the room temperature.
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Figure A3-1. A ELISA plate map used for the analysis of immunoglobulins. The standards occupied column 1, 5, and 9.

**Assay procedures:**

1) Add 100 μL capture antibody solution to each well and incubate for 60 min in room temperature. The plate was covered during incubation using an adhesive plate cover strip.

2) After incubation, aspirate the Capture Antibody solution from each well and wash 3 times with the automated wash machine (EL404, Micorplate autowasher, BIO-TEK Instruments, Inc., Winoski, VT) and then pat onto paper towel to remove residual moisture.

3) Add 200 μL of Blocking (Postcoat) Solution to each well and incubate 30 minutes.

4) After incubation, remove the Blocking (Postcoat) Solution and wash each well 3 times as mentioned above.

5) Add 100 μL of standard or sample solution to the assigned wells and incubate for 60 minutes. After incubation, remove samples and standards and wash 3 times.
6) Add 100 μL HRP Conjugate Diluents to each well and incubate 60 minutes. 
   After incubation, remove HRP Conjugate and wash each well 3 times.
7) Add 100 μL of substrate solution to each well and incubate plate 30 minutes.
   Do not wash the plate after incubation.
8) To stop the TMB reaction, add 100 μL of 2 M H₂SO₄ to each well.
9) Use the microtiter plate reader (VERSAmax Microplate Reader, MDS
    Analytical Technologies, Inc., Sunnyvale, CA), located in the Agronomy
    Laboratory, Department of Plant and Soil Science, University of Kentucky, to
    read the plate at the wavelength of 450nm. The plates were read within 30 min
    after the stop solution was added to the wells.

Calculation of results:

The example of the standard curve is presented in Figure A3-2. The amount
of pig immunoglobulin in each sample was determined by interpolating from the
sample absorbance value using the standard curve and multiply back to the dilution
rate that sample was used. Each sample was using the equation of the standard curve
obtained from the same plate.
Figure A3-2. An example of the standard curve used to derive the unknown IgM concentrations determined by the ELISA.

\[ y = 230.16x^2 - 48.511x + 27.601 \]

\[ R^2 = 0.9934 \]
References


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Professional positions

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