

THE EFFECT OF INCLUDING CAPSAICIN AND GUT MICROBIOTA FEED
ADDITIVES ON GROWTH PERFORMANCE OF NURSERY PIGS

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Chapter 1 – Literature review

Introduction

The growing global population, mainly of middle class of consumers, is challenging the food industry with an increasing demand for safe, nutritious and affordable food products. This will increase demand for meat, milk, and eggs, with pork being the most widely consumed animal-source protein in the world (Alexandratos and Bruinsma, 2012; McGlone, 2013). In this scenario, agricultural production needs to increase by 70 percent (nearly 100 percent in developing countries) by 2050 to cope with a 40 percent increase in world population (Robinson et al, 2011). However, reaching this 70 percent increase in animal-derived food product output will require excess use of natural resources such as land, water, and fisheries as well as increase the pressure on the environment due to potential pollution from manure. Therefore, it is necessary to develop and implement new technologies in the food production chain to produce food more efficiently and economically to meet this increasing demand while conserving valuable environmental resources (Davis et al., 2016; Hanjra and Qureshi, 2010).

Pork production systems evolved from an extensive pasture-based system to specialized environmentally controlled confinement buildings transforming it in a complex industrial process with significant economic and cultural value (McGlone, 2013). These intensive pork production systems require implementing management practices and feeding programs that achieve high efficiency and productivity. However, achieving optimal efficiency in pork production is being challenged by many factors such as environmental issues, public health concerns, disease outbreaks and a global increasing demand for protein. In addition, there are numerous other

efficiency and productivity challenges in the operation of modern swine production systems that must be overcome.

One of the greatest determinants of achieving high efficiency of pork production is enabling a successful weaning transition. Weaning is a stressful period in the lifecycle of pigs and can limit the capability of pigs to reach their maximum genetic potential for lean growth (King and Pluske, 2003). Health and growth performance of pigs after weaning depends mainly on a sufficient intake of nutrients. However, weaned piglets are usually not well adapted to consume solid feed, which frequently results in a prolonged period of fasting leading to health and welfare problems (Bolhuis et al., 2009). Low feed intake after weaning results in more variability, less weight gain, and often weight losses (Bruininx et al., 2001; Pajor et al., 1991). Consequently, this stressful transition may cause malfunctions at the gastrointestinal level such as intestinal villous atrophy, microbiota imbalance and diarrhea, creating an opportunity for enteric pathogens to cause digestive disorders (Pluske et al., 1997; King, 2003, King and Pluske, 2003; Madec et al., 1998; Vente-Spreuwenberg and Beynen, 2003; Langendijk et al, 2007; Castillo et al., 2007). Therefore, finding ways to ameliorate the impact of weaning stress on productivity of pigs is imperative, and various feed additives may provide substantial benefits to reduce the negative health and growth performance effects commonly observed during the weaning transition, but their effectiveness may be limited if feed intake is low and delayed in newly weaned pigs (Langendijk et al. 2007).

For several decades, the addition of subtherapeutic, growth promoting levels of antimicrobials to swine diets was a common practice to increase growth rates and feed efficiency under suboptimal health production conditions. The term antibiotic

growth promoters (AGPs) have been used to describe any drug that can kill or inhibit pathogenic bacteria when it is administered at subtherapeutic doses. These AGPs may improve growth performance by remodeling gut microbiota and decreasing the impact of potential bacterial infections in the animals (Dibner and Richards 2005). Although AGPs have been widely used for many years, their mode of action is poorly understood (Helm et al., 2019).

However, antimicrobial resistance (AMR) is a public health concern of increasing importance during the last decades and needs to be addressed by using antimicrobials in food animal production more strategically and in a responsible manner because AMR in humans is linked with AMR in animal populations (Woolhouse et al., 2015). Antimicrobial resistance was recognized after penicillin discovery for two types of bacterial strains including 1) those that are out of the spectrum of the antibacterial (naturally resistant bacteria), and 2) those that acquired the ability to survive and multiply in the presence of some antimicrobials and having consequences for human health (Acar and Moulin, 2006).

Jensen (1998) showed that bacteria which were exposed to low doses of antimicrobials over a long period of time, including those used to treat human diseases, can result in the development of resistance which prevents effective treatment with the related classes of antimicrobials in humans. Furthermore, Gassner and Wuerthrich (1994) observed that the presence of chloramphenicol metabolites in meat was associated with aplastic anemia in humans. An example supporting Jensen's observation involved chickens consuming low doses of oxytetracycline in feed, the presence of multi-drug resistant *E. coli* was isolated from feces, and discovery of their presence on nearby farms which were not using antibiotics (Cogliani et al., 2011).

Nevertheless, bacterial resistance does not always occur, such as *Streptococcus pyogenes* remaining sensitive to penicillin after several years of clinical use, and typhoid rates of infection and cure have not changed significantly since the AGP ban in EU (Hughes and Heritage, 2004). Typhoid is caused by *Salmonella typhi*, and it is known that the use of AGPs may increase the resistance to drugs in bacteria of this genus. The most common bacteria genus associated with resistance are *Salmonella*, *Campylobacter*, *Escherichia coli* and *Enterococci*, which are likely to be transmitted from animals to humans (Hughes and Heritage, 2004).

As a result, the use of AGPs in animal feeds is considered a serious threat for public health and is the one of the reasons that consumers are looking for foods based on animal protein that were produced without AGPs. The controversy around the use of AGPs in animal feed increased during the last decades finally resulted in its ban in the Europe Union (EU), the United States (US) and many other countries around the world (Ohimain and Ofongo, 2012).

The intensive use of antimicrobials in human medicine has imposed a big selection pressure to previously sensitive bacteria to code for resistance to antibiotics (Hughes and Heritage, 2004). There is a qualitative and quantitative gap in the real understanding of the antibiotics that are responsible for the bacteria resistance (Singh and Bhunia, 2019). It is well known that pharmaceutical industry waste, medicated feed, and animal and human fecal waste are the main selection pressure factors for antibiotic resistance. Furthermore, it has been projected that antibiotic resistance will cause 700,000 deaths each year by 2050 (Singh and Bhunia, 2019). However, antibiotics were approved by the U.S. Food and Drug Administration (FDA) to be used in food-producing animals to treat sick animals, to control herds where some

animals are sick or to prevent disease in health challenged animals. In these cases, antibiotics might be used at therapeutic doses and with veterinary oversight (CDC, 2020).

The use of AGPs were initially banned by the Swedish Parliament in 1986. This resulted in only a slightly decrease of growth rates in calves, turkeys and fattening pigs with no major increases in mortality. Nevertheless, outbreaks of necrotic enteritis on broiler chicken drove the efforts to improve feed and housing. Weaned pigs were also affected with increased scouring and mortality (Wierup, 2001). In an attempt to overcome these effects, efforts were focused on housing and hygiene by implementing “all in, all out” Australian model of pig production. With these changes, animal welfare was improved overall. The outcome of the model is a more expensive production system but with the possibility to have modern farming without the use of antibiotic growth promoters. This industry decreased the use of AGP by 55% in the last 13 years, which has resulted in a relatively low prevalence of antimicrobial resistance that has been maintained in the country (Wierup, 2001).

As a result of banning the use of AGP’s in animal feeds, numerous types of feed additives have become commercially available and used in diets for weaned pigs as potential replacements for antibiotic growth promoters (AGP) to enhance growth and health post-weaning. There are many various types of feed additives available, but some of the most commonly used in nursery diets are pharmacological levels of zinc and copper, prebiotics, direct fed microbials (DFM), essential oils, and plant extracts.

Plant extracts

General benefits of therapeutic plant extracts

Plant extracts are the main compounds of odor and color of plants and have two different forms as feed additives; solid powder and water insoluble essential oils commonly extracted through solvent extraction, maceration, cold pressing, or steam distillation (Liu et al., 2018). These compounds do not supply nutrients to pig diets but have many biological activities of benefit for pig health and productivity. Some of these substances (secondary plant metabolites) are alkaloids, plant phenolics, terpenes and terpenoids, with the plant phenols considered the most important because of their antioxidant, antiviral, antimicrobial, anti-inflammatory and immunostimulant properties (Tedesco, 2001).

Several plant extracts have been shown to be effective in improving pig health by limiting the impact of pathogens such as gram-negative and gram-positive bacteria including *Escherichia coli*, *Salmonella spp.*, *Klebsiella spp.*, *Proteus*, *Bacillus*, *Clostridium*, *Mycobacterium* and others (Hammer et al., 1999; Dorman and Deans, 2000; Wong et al., 2008). Phenolic compounds may limit the infectivity of pathogens by disturbing their enzyme systems and decreasing virulence (Xu et al., 2008, Burt et al., 2007, Ankri and Mirelman, 1999). Another mechanism whereby plant extracts can limit the impact of pathogens is by limiting the effects of infection on the host. For example, anti-inflammatory and antioxidant effects were identified in *in vitro* models (Liu et al., 2013). Some plant extracts inhibit proinflammatory cytokines (Lang et al., 2004; Lee et al., 2005; Tung et al., 2008; Liu et al., 2012) partially mediated by blocking the NF-kB activation pathway (Jobin et al., 1999; Lee et al., 2005; Choi et al., 2007). Herbs also have antioxidant properties. For example, carvacrol reduces

DNA lesions in hepatocytes and testicular cells exposed to H₂O₂ (Slamenova et al., 2008). Also, some plant extracts have demonstrated an antioxidant function, acting as a hydrogen donor to the peroxy radicals during the lipid oxidation (Farang et al., 1989; Djeridane et al., 2006).

The most common beneficial effect of herbs and plant extracts may be its digestive stimulant action. Ancient empirical knowledge of these benefits has been recently demonstrated with experimental research. Plant extracts coming from garlic, fenugreek, curcumin, onion, mint, black pepper, cinnamon and capsicum among others and their active components are able to activate the three peripheral sensing mechanisms (smell, taste and oral somatosensing) found in the oronasal cavities (Tominaga and Julius, 2000; Platel and Srinivasan, 1996; Srinivasan, 2007). This sensitization predisposes the gastrointestinal tract to receive food and stimulates digestive secretions and gut motility (Katschinski, 2000; Laugerette et al., 2005). Plant extracts are mainly absorbed in the stomach (80% in case of capsaicin) and in the proximal portion of the small intestine (Kawada et al., 1984). These bioactive compounds are mostly metabolized and eliminated by the kidneys resulting in a low likelihood of toxicity (Brenes and Roura, 2010).

A commercial blend of plant extracts (carvacrol, cinnamaldehyde and capsicum oleoresin) has been approved in the European Union as the first botanical feed additive for improving growth performance in livestock (Liu et al., 2018). However, the use of plant extracts in livestock feeding requires more research studies in order to provide stronger evidence of their benefits because many inconsistent growth performance results have been reported (Tedesco, 2001, Baydar et al., 2004, Sokmen et al., 2004, Dundar et al., 2008, Liu et al., e 2012; 2013a, b; 2014a, b). Data

reviewed by Rodehutscord and Kluth (2002) showed a wide range in responses from growth depression to improvement in production performance in swine (Windisch et al., 2008). Results from feeding diets containing essential oils have generally shown reduced feed intake with no change in body weight gain which leads to a better gain to feed ratio when used in poultry diets (Brenes and Roura, 2010). This variability may be caused by several conditions such as animal handling, diet digestibility, feed intake, health status, environmental conditions, feed processing, nutrient density, inclusion rate of active principle, (Zhai et al., 2018). Extraction methods, plant maturity, and harvesting time may influence the effectiveness of essential oils as well.

Use of plant extracts as enhancers of health and performance of pigs

Several studies have been conducted using phytogetic products to measure their impact on health and growth performance of pigs (Windisch et al., 2008). Oregano (*Origanum vulgare*) extracts are one of the most studied phytogetic products and have been evaluated in all stages of the lifecycle of pork production. Sows fed diets containing oregano had improved reproductive and litter performance compared with sows fed the control diet including greater farrowing rate, number of liveborn piglets and lower stillbirth rate, sow mortality and sow culling rate (Allan and Bilkei, 2005), However, no beneficial effect from feeding oregano extract to sows was observed in another experiment (Ariza-Nieto et al., 2011). Sads et al. (2003) observed an increase on growth performance and less disease incidence when pigs were fed diets containing oregano, but no benefits were found in other pig studies (Manzanilla et al., 2004; Neill et al., 2006; Nofrarias et al., 2006). A positive impact on the gut microbiota by increasing the *Lactobacilli* to *Enterobacteria* ratio, was reported by Manzanilla et al. (2004) and Nofrarias et al. (2006) when a mixture of oregano,

cinnamon and Mexican pepper were added to diets of weaned pigs (Liu et al., 2018). Villus height and crypt depth in small intestine were increased, and lower numbers of intra-epithelial lymphocytes were observed after a supplementation of carvacrol and thymol in pig diets (Michiels et al., 2010). Nonetheless, Kosińska and Andlauer (2013) found some unforeseen implications of using plant extracts. Extracts of sweet pepper, marigold, ginger, sage and green tea extract, lectin, and capsaicin may disrupt functions of tight junctions' assembly reducing transepithelial electrical resistance (TEER) and increased the permeability *in vitro*. This evidence may suggest avoiding the use of some plant extracts under digestive disease conditions.

These findings may support our premise that plant extracts may be useful to replace antibiotic growth promoters, but results have been variable and influenced with many other external factors such as health status of the herd, animal management, diet, age and microbiota composition. Thus, more studies need to be addressed in order to have stronger evidence about plant extracts beneficial effects on livestock production.

Capsaicin

Capsaicinoids are extracts from chili pepper. These compounds may promote health and growth of pigs by improving their health, improving digestion of nutrients, and increasing feed intake. Capsaicin (Figure 1) is the most common of the five natural capsaicinoids (botanical pungent extracts) from chili pepper, which belongs to the genus *Capsicum* (Bennett et al., 1968). This genus from the “new world” has been used for the last 6,000 years with great economic importance for food and spices (Moscone et al., 2006). This compound was first extracted in impure form in 1816 by Christian Friedrich Bucholz who named it “capsicin”, and later in 1876, John Clough

Thresh isolated it in almost pure form.

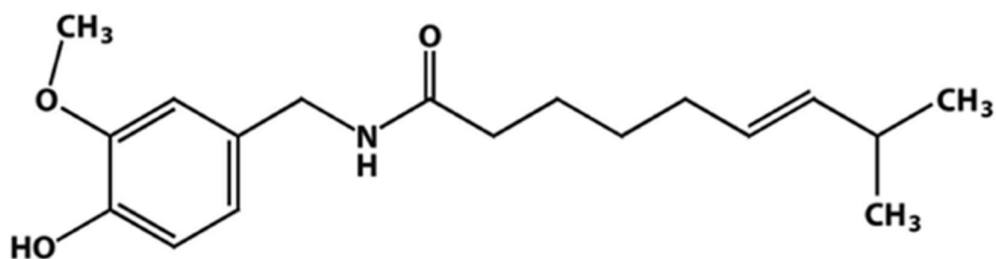


Figure 1.1 Molecular structure of 8-methyl-N-vanillyl-6-nonenamide (Capsaicin) C₁₈H₂₇NO₃ (National Center for Biotechnology Information, 2005).

There are beneficial effects of capsaicin in human and animal diets including anti-obesity, anti-inflammatory, antioxidant, and anti-cancer properties (Zimmer et al., 2012; Clark and Lee, 2016; Zheng et al., 2017). These benefits may be due to the non-narcotic analgesic, voltage-gated sodium channel blocker, and a TRPV1 agonist property of capsaicin, which is also a neuropeptide selective releasing agent for primary sensory peripheral neurons (National Center for Biotechnology Information, 2005).

The effects of capsaicin on health can be local at the gut level or systemic because capsaicin is passively absorbed in the stomach and in the upper portion of the small intestine, with more than 80% efficiency (Kawada et al., 1984). Capsaicin-sensitive afferent neurons in the stomach may be activated, which play a central role in the neuronal mechanisms of the stomach (Holzer, 1998). These afferent neurons regulate various gastric functions such as secretion, mucosal blood flow, motility, and modulate the mucosal integrity (Takeuchi et al., 1991; Holzer, 1998). These functions may help pigs after weaning when use to show low feed intake and digestive capacity with a negative impact on intestinal permeability and intestinal epithelial tissue regeneration (Pajor et al., 1991; Cera et al., 1998; Bruinix et al., 2001; Liu et al.,

2018). This compound can also be used to manipulate substance P and other tachykinins (only experimentally), control induced mucositis in special treatments like chemotherapy and radiotherapy and serve as a peripheral nerve pain reliever (National Center for Biotechnology Information, 2005).

Dietary capsaicin and other spice compounds may enhance lipid digestion and absorption when consuming diets high in lipid content by stimulating secretion of bile salts and stimulating the activity of pancreatic lipase (Prakash and Srinivasan, 2012). Digestibility of lipids in nursery pigs is low, especially during the first weeks post-weaning (Cera et al. 1988). This low digestibility of lipids also depends on the types and sources of fatty acids where saturated fatty acids are less digestible than polyunsaturated fatty acids (Kerr et al., 2015). Therefore, addition of capsaicin may be beneficial to young pigs post-weaning in order to improve lipid digestibility and increase growth of pigs by increasing energy digestibility. However, there are no studies that have demonstrated that capsaicin increases digestibility of lipids in nursery pigs or that affect lipid mediated physiological pathways.

The efficacy of capsaicin may depend on the purity of the product, which may be related to multiple factors including extraction methods. Historically, capsaicin extraction was performed using organic solvents, microwave-assisted extraction procedures, and ultrasound-assisted extraction among others (Chinn et al., 2011). However, the efficacy of these processes varies based on pepper varieties, their parts, and pre-extraction processing (Chinn et al., 2011; Nazari et al., 2007; Yue et al., 2012). Extracting by solvent procedures begins with the separation of seeds and shells from the whole pepper, followed by oven-drying at 65°C (always under 70°C to minimize losses through volatilization) for 24 hours, and freeze drying for 4 days with

a condenser refrigeration of -80°F, shelf temperature of +80°F, and chamber pressure of 500 atm. This preparation must be extracted with a biomass solving loading of 15% based on pepper moisture content. Homogenization of this mixture needs to be done in a shaking water bath at 50°C (Chinn et al., 2011). Soxhlet extraction requires use of a finely ground sample with an acetone extraction until the tissues are decolorized, which occurs in about 2 h. This extract is then evaporated to dryness using a rotary evaporator at room temperature. Ultrasonic extraction of dried samples must be sonicated at room temperature with the addition of acetonitrile for 1 h, according to Karnka et al. (2002). After that, the sample needs to be evaporated to dryness with a nitrogen stream. Microwave assisted extraction is performed using a microwave accelerated reaction system capable to sustain a temperature and pressure up to 200°C and 200 psi (Nazari et al., 2007).

Effects of feeding capsaicin on growth performance and gene expression of pigs

As described previously, there are limited studies related to the effects of feeding diets containing capsaicin on health and growth performance of pigs, and most studies evaluated capsaicin in combination with other plant extracts. Therefore, these studies were confounded when attempting to understand the direct effects of capsaicin itself. However, a few studies have evaluated the sole addition of capsaicin to swine diets.

A study conducted at Chiang Mai University (Thailand) with 24 newly weaned pigs during 35 days concluded that 5 ppm of capsaicin in weaned pig feed had improved their average daily feed intake and feed conversion ratio compared with control group (without capsaicin) and also compared with a colistin sulphate feed

additive treatment (100 ppm). In this experiment capsaicin had no effect on inhibiting *E. coli* proliferation and diarrhea score (Rujirapong et al., 2010).

In another study, Liu et al. (2013a, b) found that 64 weaned pigs infected with a pathogenic F-18 *Escherichia coli* strain and fed a diet containing 10 ppm of capsicum oleoresin, reduced diarrhea and inflammation caused by *E. coli* infection compared with the control treatment. There were other 2 plant extract treatments in the same study (10 ppm garlic botanical and 10 ppm turmeric oleoresin), and all plant extract treatments had the same beneficial effects on health and immune responses compared to pigs fed the control diets. Furthermore, 64 weaned pigs infected experimentally with porcine reproductive and respiratory syndrome virus (PRRSV) and fed diets containing 10 ppm of capsicum oleoresin, 10 ppm garlic botanical, or 10 ppm turmeric oleoresin reduced the adverse effects of PRRSV by improving the immune responses of pigs. Moreover, the turmeric oleoresin treatment improved feed efficiency of pigs challenged with PRRSV compared with the other dietary treatments.

Capsaicin may also have a positive effect on the passive immunity in piglets. Sows fed gestation and lactation diets containing 10 ppm of capsaicin and previously vaccinated against Aujeszky's disease, had increased immunoglobulin G concentrations in serum of the piglets until 28 days of age (Paraksa, 2011). This improvement may be associated with the antioxidant effect of carotene and vitamin C which are present in capsicum crude extract (Chairattanasat, et al., 2009).

There are also some studies that have evaluated gene expression related to the use of capsaicin in pig diets that has been associated with gut mucosa and immune response. Liu et al. (2014a, b) observed also that feeding capsicum oleoresin to

nursery pigs altered the expression of 490 genes (280 up regulated and 210 down regulated) compared to pigs fed the control diet. Many of these genes were related with integrity of membranes, tight junctions (enhancing gut mucosa health), and immune response in normal health conditions. In weaned pigs infected with pathogenic F-18 *Escherichia coli*, the expression of 240 genes was altered and compared with infected capsaicin fed pigs which altered the expression of 52 genes that were mainly associated with counteracting *E. coli* effects. Lastly, feeding a diet containing a combination of liquid methionine and capsaicin to weaned pigs had a positive effect on feed conversion, and increased villous height in the small intestine which may suggest an increased nutrient absorption capacity of enterocytes (Jarupan et al., 2018).

Although studies have shown beneficial effects in the GI tract and improvements in growth performance in weaned pigs fed diets containing several plant extracts including capsaicin, results among studies are inconsistent. In addition, there is evidence that dietary capsaicin may improve passive immunity and alter gene expression of nursing pigs through milk and colostrum intake. However, no studies have been conducted to evaluate subsequent growth performance effects and potential changes in gene expression of weaned pigs from sows fed plant extracts, and specifically capsaicin, and continuing with capsaicin included in their diets after weaning.

Direct fed microbials and prebiotics

Gut microbiota modifying feed additives as possible alternatives

Gut health is partially determined by the microbiota composition. Some bacteria genus may be essential for the microbial community to maintain it in balance

(Roberfroid et al., 2010; van der Aar et al., 2017). Physiological functions of intestinal flora may contribute to overall health status, and an imbalance in gut microflora can be detrimental (Holzapfel, 1998). A “balanced” microflora is essential for efficient digestion of food, produces some vitamins, stimulates immune system, and produces protective enzymes and short chain fatty acids (Holzapfel, 1998). A mature microbial ecosystem also utilizes available nutrients and fill all environmental niches preventing pathogenic bacteria (Jin et al., 1997).

Microbial colonization starts during delivery from birth canal of the mother to the environment, where *E. coli* and *Streptococcus* first predominate, but during suckling, there is an increase in numbers of *Bifidobacterium* to the detriment of the other microbial populations (Jiang et al., 2019). This strongly suggests that the diet will affect the microflora composition. As pigs increase in age, a succession of microbial species colonizes the gut which increases in complexity until a stable population becomes established (Lu et al., 2003). To achieve a good performance of pigs after weaning it is necessary to support a good development of a healthy intestinal tissue, overall intestinal immune system and a lactic acid bacteria colonization (Stein and Kil, 2006). After weaning, lactic acid-producing bacteria concentration decreases in the GI tract, suggesting that supplementing weaned pig diet with *Lactobacilli* spp. as direct-fed microbials (DFM) may be beneficial (Stein and Kil, 2006).

Many strategies have been developed to maintain this essential balance of the gut microbiome, such as competitive exclusion of selected microbial species which has had varying degrees of success under field conditions (Callaway et al., 2008). Feeding live microorganisms found in DFM, which are also known as probiotics, are

thought to provide a positive impact on gut health by maintaining optimal balance of the microbial population in the host which may improve nutrient absorption and immune response (Dhama et al., 2008). In a similar manner, prebiotics (indigestible carbohydrates and some bacteria and fungi strain extracts) are utilized by beneficial bacteria to stimulate their growth and create an adverse environment for pathogens (Dhama et al., 2008; Guan, 2017; Sanchez, 2019). Prebiotics may also reduce biogenic amines that cause intestinal injury and inflammation (San Andres et al., 2019). Other feed additives, such as zinc oxide and copper sulfate, provide beneficial effects to the GI tract including improvements in villus morphology, increased crypt depth, regeneration of injured intestinal epithelial tissue, reduction of intestinal permeability, and a reduction of post-weaning scouring, especially in newly weaned pigs (Liu et al., 2018). However, these additives are restricted in many countries because of their harmful effects on the environment caused by high concentrations of Zn and Cu in manure that is used as fertilizer when applied to soil (Jondreville et al., 2003). Therefore, direct fed microbials (DFM) and prebiotics may be more appropriate alternatives to reduce utilization of antibiotics, zinc, and copper in pig diets if they provide similar growth and health responses (Choct 2009; Williams et al. 2001; Cheng et al. 2014).

Direct fed microbials

Live microorganisms which provide health benefits to the host when administered in adequate amounts are considered probiotics (FAO, 2001). Probiotics are also referred to as “direct-fed microbials.” The U.S. Food and Drug Administration (FDA) defines direct fed microbials as “...products that are purported to contain live (viable) microorganisms (bacteria and/or yeast).” (Quigley, 2011).

Generally recognized beneficial microorganism species are the *Bifidobacterium* spp. and *Lactobacillus* spp. (Gibson and Roberfroid, 1995). These species are beneficial when added directly to the diet as DFM or when their presence and growth in the gastrointestinal tract is enhanced by prebiotics.

Foods fermented by lactic acid bacteria had been consumed by humans for a long time with several health promoting effects, which may be the result of metabolites of the bacteria in the food products, or the structural components of the bacteria, or both (Ouweland, 2002). These health promoting effects may be dependent on the type of bacterial strain (Ouweland, 2002). The word “probiotic” is Greek, which means “for life”, was first used by Lilley and Stillwell in 1965 when they described a secretion of a microorganism which enhanced the growth of another, but it was Parker in 1974 who first used the term probiotic to name “organisms and substances which contribute to intestinal microbial balance” (Fuller, 1992). Therefore, direct fed microbials are defined as live microbial feed additives which may improve health status by decreasing the possibility of clinical or subclinical health challenges (Roberfroid et al., 2010; van der Aar et al., 2017). This occurs by lowering gut pH by lactic acid producing bacteria and increasing the production of short chain fatty acids (SCFA) through bacterial fermentation of indigestible carbohydrates, which may reduce pathogenic bacteria population (Smiricky-Tjardes et al., 2003).

Table 1 shows a partial list of the most common direct fed microbials (DFM) used for human and animal consumption (Goldin, 1998).

Lactic acid bacteria have an antagonistic effect on the growth of pathogenic bacteria through the production of bactericidal substances such as organic acids (lactic and acetic acids) and bacteriocins (Yang et al., 2015). *Bacillus*-based DFM are

thermostable and able to survive at low pH because they are spore-forming (Liu et al, 2018), and their capacity to produce extracellular fiber-degrading enzymes may improve nutrient digestion and utilization. In contrast, lactic-acid producing bacteria are not spore-forming and their viability after high temperature feed processes (such as pelleting or extrusion) is a concern (De Lange et al., 2010). Competitive exclusion is another mechanism of probiotics to enhance the protection towards pathogenic bacteria by occupying environmental niches and utilizing available nutrients (Jin et al., 1997). In addition, DFM products may improve performance of challenged animals by increasing digestive enzyme activity, decreasing ammonia production, enterotoxin neutralization, and stimulation of immune system (Jin et al., 1997).

Table 1. Micro-organisms used as probiotics for humans and animals.

Bacteria

Lactobacillus

acidophilus
plantarum
casei
rhamnosum
rhamnosus GG
delbrueck subsp. Bulgaricus
reuteri
fermentum
brevis
lactis
cellobiosus

Bifidobacterium

bifidum
infantis
longum
thermophilum
adolescents
animalis

Streptococcus

lactis
cremoris
alivarius subsp.
thermophilus
intermedius

Leuconostoc

Pediococcus

Propionibacterium

Bacillus

Enterococcus

Enterococcus faecium

Yeast and molds

Saccharomyces cerevisiae

Candida pintolopesii

Aspergillus niger

Aspergillus oryzae

There are several studies about probiotics or direct fed microbial (DFM) on pig performance as well. The addition of *Bifidobacterium globosum* (a lactic-acid producing DFM) to weanling pig corn-soybean meal-based diets improved average daily gain (ADG) and average daily feed intake (ADFI) but did not affect gain efficiency (G:F), immune response, or pH of intestinal contents. The same DFM inclusion was maintained through growing-finishing phase and did not affect growth performance and carcass characteristics (Apgar et al., 1993). In more recent studies *Bifidobacterium lactis* supplemented to a weanling pig's diet increased gut barrier function (Lewis et al., 2013). However, when 60 kg pigs were fed either a corn-soybean meal diet without or with DFM, no differences in the ATTD of dry matter, acid detergent fiber, and neutral detergent fiber were observed (Kornegay and Risley, 1996). Nevertheless, ADG, G:F, and carcass quality were improved with increased dose and duration of DFM addition to wean-to-finish pigs' diets (Alexopoulos et al., 2004).

Bacillus sp. were also evaluated as DFM on pig's performance. Diets containing distillers dried grains with solubles (DDGS) and supplemented with a *Bacillus*-based DFM had increased available dietary energy with subsequent greater growth performance in newly weaned pigs and in growing-finishing pigs as well (Owusu-Asiedu et al., 2014; Jaworski et al., 2014). Another study using a *Bacillus*-based direct-fed microbial supplemented to sows' diet obtained an improvement on weaning weight and litter ADG, and more pigs were weaned compared to the control group. Gastrointestinal microbiota of piglet was affected by the *Bacillus*-based DFM supplementation to the dam. Piglet mortality was not different between treatments (Baker et al., 2013).

Prebiotics

Prebiotics are mainly indigestible oligosaccharides but also substrates that are selectively utilized by gut microbiota conferring health benefits (Gibson and Roberfroid, 1995; Hess, 2014). These food ingredients act by stimulating the activity of some beneficial bacteria in the large intestine improving host health because of its easy fermentable capacity and reduction of luminal pH (Gibson and Roberfroid, 1995; de Lange et al., 2010; Knudsen et al., 2012; Gibson et al., 2011). Prebiotics also reduce biogenic amines that cause intestinal injury and inflammation (San Andres et al., 2019).

Indigestible carbohydrates are considered prebiotics if they can be degraded by the gut microbiota (mainly bifidobacteria) used in the production of short chain fatty acids (SCFA) as degradation products (Liu et al., 2018). Bacterial fermentation of polysaccharides and oligosaccharides in the large intestine produce short chain fatty acids (SCFA) as end products which provide benefits on colonic health. The SCFA production is affected by microbiota and substrates in the colon (Cummings and Macfarlane, 1991).

There are only a few important groups of indigestible carbohydrates that serve as prebiotics and provide beneficial effects on human and animal health. These include fructo-oligosaccharides, transgalacto-oligosaccharides, mannan-oligosaccharides, lactulose, and inulin, which naturally exist in food but in low amounts (Gibson and Roberfroid, 1995; de Lange et al., 2010; Knudsen et al., 2012). Prebiotics also include substrates that are selectively utilized by gut microbiota to confer health benefits, including arabino-xylans, xyloglucans, and resistant starch (Knudsen et al., 2012). Because of their potential health benefits and safety, these

products appear to be good candidates for health enhancement as a replacement or in association with probiotics and are produced commercially for use as functional ingredients and supplements by hydrolyzing polysaccharides or disaccharides, being most of them synthesized or isolated from plant and algae polysaccharides (Smiricky-Tjardes et al., 2003; Saad et al., 2013; Wu et al., 2017; Liu et al., 2018; Davani-Davari et al., 2019; Lockyer and Stanner, 2019).

Dietary fiber is important for optimal health, where high fiber diets may reduce the risk of some chronic diseases due to its effect on gut function and microbiota, cholesterol, and glycemic levels (Lockyer and Stanner, 2019). Results from several studies have shown that fiber exclusion from diets result in the reduction of *Bifidobacterium* and *Lactobacillus* species with unknown long-term health implications (Lockyer and Stanner, 2019). However, *Bifidobacterium*, *Lactobacilli*, and *Eubacteria* in the gastrointestinal tract may improve the health of the animals, and decrease the risk of diseases (Roberfroid et al., 2010; van der Aar et al., 2017).

Other products with prebiotic functions

While the majority of prebiotics are carbohydrates of plant origin, it is well recognized that other nutrient and chemical entities may have prebiotic effects and consequently new definitions of prebiotics were modified to include additional elements that help to define a prebiotic (Gibson et al., 2017). One product that has prebiotic benefits under the new definition are the fermentation broth or extracts from fungi such as *Aspergillus oryzae* or bacteria such as *Lactobacillus acidophilus*.

Aspergillus species, including *A. oryzae*, *A. sojae* and *A. luchuensis*, are known in Japan as koji molds which have an important role in Japanese fermented products such as sake (rice wine), shoyu (soy sauce), miso (soybean paste) and

shochu (distilled beverage), but specifically, *A. oryzae* is well known for its high capacity to produce amylases and proteases at industrial levels and are also noted for their capacity to secrete enzymes in solid-state rather than liquid cultures (Yoshimi et al., 2016). However, other *Aspergillus* genus species are not beneficial, and some species are aflatoxin producers which is a known natural carcinogenic substance (Yoshimi et al., 2016). *A. fumigatus* is the major cause of invasive aspergillosis causing severe symptoms in immunosuppressed patients (Yoshimi et al., 2016). In contrast, *Aspergillus oryzae* (fungi) and *Saccharomyces cerevisiae* (yeast) are considered probiotics, but their fermentation extracts are described as prebiotics since *A. oryzae* produces cellulases, hemicellulases, and esterases in an aerobic environment, this may increase microbial activity and fermentation (Varel and Kreikemeier, 1994).

There is very limited information about the effect of *Aspergillus oryzae* fermentation extract (AFE) on nursery pig's performance, nevertheless some studies were done in sows. *Aspergillus oryzae* fermentation extract (AFE) was added on gestation standard corn-soy bean meal of 36 sows in a dose of 45g/day. Sows performance and colostrum quality was measured. Treatment group loss less weight than the control group but immunoglobulin levels and sow performance showed no significant differences (Hess, 2014). Another study was performed with AFE provided as top dress to sows and to their offspring in feed after weaning. Treatment showed no significant effect on feed intake, body condition, piglet weaning weight, or return to estrus of sows but a significant negative effect on piglet gain. After weaning, pigs fed with AFE in diet showed no significant effect on growth performance (Jackson et al., 2006). Furthermore, Moeller et al. (2016) fed sows with AFE since day 70 of gestation and measure sow performance. In this study, no significant

differences in number of pigs born alive and litter birth weight were found for treatments or parity but treated sows tended to wean heavier litters. Colostrum and milk quality were not different for IgM, IgG or IgA, blood lactate and L-lactate were less at weaning in treated sows.

Lactobacillus acidophilus fermentation product (LAFP) is considered a prebiotic and contains some metabolites such as organic acids, peptidoglycans, proteins and peptides (Sanchez et al., 2019). This product had beneficial effects in the performance of weaned pigs and the population of beneficial gut bacteria (Sanchez et al., 2019). Feeding LAFP to pigs, provided health benefits when exposed to heat stress (Guan et al., 2017), increased average daily weight gain, average daily feed intake, and reduced the use of injectable antibiotics (Lee et al., 2016). Studies in humans with LAFP, found positive effects on the immune system (Schabussova and Wiedermann, 2008), and in a study in pigs improving immunoglobulin response to vaccination (Lessard and Brisson, 1987). However, the ability of LAFP to modulate inflammation or immune function is unknown (Sanchez et al., 2019).

In other studies, *Lactobacillus acidophilus* fermentation product (LAFP) was added to nursery feed and 140 newly weaned pigs were fed during 6 weeks after weaning with 4 treatments (1 control treatment and 3 different levels of LAFP). Growth performance, nutrient digestibility, fecal microbiota and noxious gas emissions were evaluated and compared among the four treatments. Average daily gain and digestibility of dry matter improved linearly with increasing inclusion of LAFP. Improvement in nitrogen digestibility associated with the inclusion of LAFP was observed as well. Inclusion of LAFP changed the proportion of fecal *Lactobacillus* and *E. coli* on day 42 (Lan et al., 2016). In a different study, pigs with

chronic K88+ (F4) *Escherichia coli*, improves health status and reduces injections and mortalities when fed with a combination of 1 kg/MT. of LAFP, carbadox and zinc oxide (Probst Miller et al., 2016). In addition, two more studies were performed by Guan et al. (2017) comparing the use of LAFP and zinc oxide at pharmacological levels. In the first experiment, 288 newly weaned pigs were evaluated for 22 days were zinc oxide at pharmacological level treatment (3000 ppm of ZnO) had no differences in performance compared to a treatment including 2 Kg/MT of LAFP in pre-starter diet (from weaning to 12 Kg. of body weight) and 1 Kg/MT of LAFP in starter diet (from 12 Kg. of body weight to the end of the study). In the second study, 48 newly weaned pigs (24 boars:24 gilts) were assigned to 4 treatments comparing the use of LAFP and zinc oxide at pharmacological level (2500 ppm) during 22 days after weaning and orally challenged with 5 mL of 8.9 Log of nalidixine resistant *E. coli*. Pigs showed lower fecal excretion of *E.coli* when 2 Kg/MT of LAFP was added in feed, but zinc oxide treatment resulted in a greater average daily gain. In a recent study, newly weaned pigs were fed with 4 dietary treatments (with and without lactose, and with and without a prototype *Lactobacillus acidophilus* fermentation product). Findings demonstrated that lactose improved feed intake, digestibility of dry matter and gross energy, and the nitrogen retention of weaned pigs. However, *Lactobacillus acidophilus* fermentation product showed slightly an increase of nitrogen digestibility (Acosta et al., 2020).

Yeast cell walls contain β - D-glucans, and there is evidence that they affect the immune system by enhancing macrophage and neutrophil function through binding their receptors, causing cytokine cascades, and increased antibody production (Kogan and Kocher, 2007; Kim et al., 2017; Molist et al., 2014). Inulin is a generic term for linear fructans that provide oligofructose as a partial hydrolysis product. Fiber

improves intestinal motility by increasing the biomass and water content of feces, but inulin has other characteristics that affect gastrointestinal functions because of their biochemical and physiological attributes rather than their physico-chemical properties. They produce SCFA in the colon, through a fermentation process, stimulating the growth of bifidobacteria, and some other beneficial genera, with significant changes in the composition of the microbiota. Several animal studies have shown the capacity of inulin-type fructans to enhance calcium and magnesium absorption and its capacity to affect the lipids metabolism reducing plasma very-low-density-lipoproteins (VLDL) particles, decreasing triglyceridaemia as a result. These characteristics may contribute to the capability of DFM and prebiotics to be possible alternatives for AGP's (Berg 1998; Kim et al. 1999; Wenk 2000).

Gilts offspring and the concern of unachieved growth potential

As a result of last decade genetic selection advances, modern high prolific sows increased litter size and light weight piglets at birth as well, which are at greater risk of preweaning mortality (Zeng et al., 2019). The rearing ability of gilts compared to older sows is also observed to be lower, however, factors such as piglet birth weight and suckling demand can affect litter gains from gilts and sows (Smits and Collins, 2009).

Gilt-derived progeny often have suboptimal growth and health status compared with offspring from older sows (Craig et al., 2017), and are usually smaller in birth weight and may have insufficient immunoglobulin protection being this identified as a major issue in pig production. (Smits and Collins, 2009). Responsible mechanisms are not fully understood, nevertheless anatomical differences existing between them after birth, are still observed at weaning (Craig et al., 2019). On

average, gilt progeny have lower weight at birth and at weaning, which can persist through the fattening period (Craig et al., 2019; Carney-Hinkle et al., 2013; Craig et al., 2017a, b). Gilt-derived pigs have also greater post-weaning medication and mortality rates compared with progeny from older sows (Craig et al., 2017). Passive immunity is provided by immunoglobulins in colostrum and is also well known that gilts have lower production colostrum yield and also lower quality, IgA and IgG concentrations are greater in colostrum from older parity dams compared with younger dams (Inoue et al., 1980; Inoue, 1981a; Klobasa et al., 1985, 1986; Craig et al., 2019). Microbial ecology is also affected by dam's parity. Gilt derived progeny has lower diversity, which is passed from the dam and influences pig's microbiome during its productive life (Tran et al., 2019). This may suggest that DFM and prebiotics could have a positive influence specially in gilt's microbiota progeny.

In summary, limited studies with inconsistent responses require additional studies to determine the potential for using DFM, prebiotics, and capsaicin on health and growth performance of weaned pigs. Furthermore, little is known about differences in growth performance responses of progeny from gilts compared with progeny from multiparous sows when fed these feed additives. Finally, effects of maternal diets supplemented with plant extracts such as capsaicin on subsequent growth performance of progeny and gene expression have not be well defined. Therefore, the studies conducted in this thesis had two main objectives. The first objective was to evaluate newly weaned pig growth performance when they and their dams were fed diets with or without capsaicin. The second aim was to evaluate the effect of the inclusion of different DFM and prebiotic products, supplemented in nursery pig diets, on their growth performance and compare growth responses between gilt and sow derived progeny.

Chapter 2 – Effect of supplementing lactation and nursery pig diets with capsaicin on growth performance and gene expression of nursery pigs

SUMMARY

The objective of this study was to determine the effects of supplementing capsaicin in diets for lactating sows and their offspring on the growth performance and gene expression of pigs post-weaning. Twenty-eight multiparous sows were fed corn-soybean meal-based diets with (n = 14) or without (n = 14) 2.5 ppm of capsaicin during a 19-d lactation period. Litters from these sows (n = 288 pigs) were weaned and assigned to 36 blocks (pens) based on maternal dietary treatment and initial body weight (**BW**) to provide 8 pigs/pen. Blocks were assigned randomly to one of two nursery dietary treatments (control or capsaicin supplemented diets) in a 2 × 2 factorial arrangement of treatments to provide 9 replications per treatment combination. A 3-phase nursery feeding program was used, where phase 1 diets were fed from weaning to 7 d, phase 2 diets were fed from 8 to 21 d, and phase 3 diets were fed from 22 to 35 d post-weaning, with or without 1.0, 1.3, and 1.6 ppm capsaicin, respectively. Individual BW and pen feed disappearance were recorded at each dietary phase change. Data were analyzed using a mixed model with the effect of nursery dietary treatment nested within sow lactation treatment, the effect of time with repeated measures, and interactions between treatments and wk post-weaning. On d-38 post-weaning, blood samples were collected from one pig in each pen (n = 36) with BW closest to the pen average for RNA sequencing and gene expression analysis. Pairwise comparisons among dietary treatment groups were used to determine differences in gene expression. There were no effects of feeding capsaicin diets to lactating sows and/or their weaned offspring on BW, average daily gain

(**ADG**), or average daily feed intake (**ADFI**) of pigs during the 35-d nursery period. However, pigs weaned from sows fed capsaicin during lactation and continuing on capsaicin diets during the nursery period tended ($P = 0.09$) to have greater gain efficiency (**G:F**) than pigs fed the other dietary treatments. Furthermore, there was an interaction ($P < 0.01$) for G:F for dietary treatment and wk post-weaning, where the magnitude of improvement was greater during the first wk post-weaning than subsequent wks. There were a limited number of differentially expressed genes among dietary treatment combinations. The greatest number of differentially expressed genes occurred in offspring from sows that were fed capsaicin during lactation compared with the offspring from sows with no exposure to capsaicin. In conclusion, the combination of feeding capsaicin to sows during lactation and to nursery pigs at weaning appears to improve gain efficiency for the first wk post-weaning and may alter gene expression to a greater extent than when capsaicin is supplemented only in the nursery diets.

Key words: Capsaicin, feed intake, gene expression, growth performance, lactation diet, nursery pigs.

INTRODUCTION

One of the greatest challenges in optimizing growth performance and health of newly weaned pigs is to overcome low and variable feed consumption during this critical transition period on commercial swine farms. Low feed intake, which commonly occurs immediately after weaning, often results in small intestine structure alterations (Pluske et al., 1997), villous atrophy (Langendijk et al, 2007), and diarrhea (Dong and Pluske, 2007). Therefore, dietary interventions that enhance feed intake, energy and nutrient digestibility are needed (Dong and Pluske, 2007). Numerous

types of feed additives are commercially available and used in diets for weaned pigs to enhance growth and health post-weaning as potential replacements for growth promoting antibiotics. Among the various types of feed additives, essential oils and plant extracts have become increasingly popular choices in nursery diets because of their capability of enhancing digestive enzyme secretions and nutrient absorption, reducing gut pathogens, providing antioxidant properties, and improving immune status (Zeng et al., 2015).

Capsaicin is a biologically active compound derived from the genus *Capsicum* (chili peppers) and has antimicrobial, anti-obesity, anti-inflammatory, antioxidant, and anti-cancer properties in human and animals when supplemented in the diets (Zimmer et al., 2012; Clark and Lee, 2016; Zheng et al., 2017). Capsaicin is passively absorbed in the stomach and the upper portion of the small intestine with more than 80% efficiency (Kawada et al., 1984). Upon consumption, capsaicin-sensitive afferent neurons in stomach can be activated, which play a central role in the neuronal mechanisms of the stomach (Holzer, 1998). These afferent neurons regulate various gastric functions such as secretion, mucosal blood flow, and motility, and also modulate the mucosal integrity (Takeuchi et al., 1991; Holzer, 1998). As a result, studies have shown that feeding capsaicin enhances digestive enzyme secretion through neurostimulatory activity (Maggi et al., 1987; Platel and Srinivasan, 1996; Maji and Banerji, 2016), and can also provide potent antioxidant activity in vitro (Kogure et al., 2002). Dietary capsaicin and other spice compounds have also been shown to enhance lipid digestion and absorption when consuming diets high in lipid content by enhancing secretion of bile salts and stimulating the activity of pancreatic lipase (Prakash and Srinivasan, 2012).

However, because many commercially available botanical extracts are mixtures of various compounds, it is difficult to differentiate the relative contribution of each compound to growth and health responses observed. For example, Ilsley et al. (2003) fed a blend of carvacrol, cinamaldehyde and capsicum to lactating sows and showed improvements in nutrient digestibility and pre-weaning piglet growth performance, but the relative contribution from capsaicin toward these improvements is unknown. Doses between 2 and 2.2 ppm of capsaicin (as a part of a blend of plant extracts) in gestation and lactation feed, showed beneficial effects on sows and their offspring performance such as better backfat thickness, high lactose content in milk, high average daily gain of piglets during lactation period among others (Ilsley et al., 2003; Matysiak et al., 2012). Positive effects on the passive immunity of piglets from nursing sows supplemented with up to 10 ppm of capsicum crude extract in their gestation and lactation diets was also reported by Paraksa (2011).

Weaned pigs fed with 10 ppm of 3 different plant extracts (capsicum oleoresin, garlic botanical, and turmeric oleoresin) were effective in increasing the expression of genes related to integrity of membranes and tight junctions, indicating enhanced gut mucosa health and increase the expression of genes associated with immune responses as well. (Liu et al., 2014). Additional studies by Liu et al. (2013a, b) showed that feeding the same concentrations of each of these 3 plant extracts enhanced immune responses and gain efficiency of weaned pigs infected with porcine reproductive and respiratory syndrome virus. Further evidence of beneficial effects on growth performance and health from feeding capsaicin supplemented diets to weaned pigs was reported by (Rujirapong et al., 2010), where feeding diets containing 5 ppm capsicum resulted in improved ADG, ADFI, and G:F compared with pigs fed diets containing no capsicum.

Any nutritional intervention or feed additive that encourages faster adaptation and greater feed consumption in newly weaned pigs is of great benefit for optimizing pig health and subsequent growth performance (Langendijk et al. 2007). It may be that some feed additives, such as capsaicin or other botanical extracts, can be supplemented in lactating sow diets to encourage faster adaptation and consumption of dry diets of their offspring immediately after weaning if these compounds are present in sow milk during the nursing period (Oostindjer et al., 2010). Although evidence for this potential benefit is limited, Charal et al. (2016) found that supplementing lactating sow diets with anise oil to lactating sows and to their offspring after weaning, may improve ADFI of pigs during first days after weaning. However, there are no studies that have evaluated the effects of feeding capsaicin to lactating sows and the subsequent effects on growth performance and gene expression of their offspring fed diets with and without capsaicin post-weaning. Therefore, the objectives of this study were to determine the potential carry-over effects of pigs nursing sows fed capsaicin diets on post-weaning growth performance and gene expression compared with offspring from sows without capsaicin supplementation but fed capsaicin only during the post-weaning nursery period. Therefore, we hypothesized that adding capsaicin to lactating sow and subsequent nursery diets may improve feed intake of weaned pigs during the critical first wk post-weaning.

MATERIALS AND METHODS

The University of Minnesota Institutional Animal Care and Use Committee (Protocol No. 1812-36585A) approved all the experimental procedures used in this study.

Facilities and animal management

This study was conducted at the University of Minnesota West Central Research and Outreach Center (**WCROC**) located in Morris, MN in two phases using a 2×2 factorial arrangement of treatments. The lactation phase involved feeding corn-soybean meal-based diets with or without 2.5 ppm capsaicin to sows during a 19-d period, followed by the nursery phase where the weaned offspring were fed diets with and without capsaicin during a 35-d post-weaning period to determine the effects of capsaicin on growth performance and gene expression.

Sow feeding

Twenty-eight multiparous crossbred sows (Large White \times Landrace; Topigs Norsvin®, Burnsville, MN) from one farrowing group were used in this study. Sows were allotted randomly to one of two corn-soy bean meal-based diets that were formulated to meet or exceed NRC (2012) requirements for lactating sows using previous sow productivity data for this research herd (Table 2.1). Fourteen sows were fed a diet with containing 2.5 ppm capsaicin (Leader® Capsaicin 2%, Leader Bio-Technology Co., Ltd., Guangzhou, China) and another 14 sows were fed the same diet but without capsaicin. All sows were housed in individual farrowing crates (1.5 \times 2.0 m) and were provided ad libitum access to experimental diets and water from the day of farrowing until weaning (19.18 ± 1.16 days). Piglets were processed according to

the WCROC standard operating procedures (**SOP**) and cross-fostered within each treatment to standardize litter weights and litter size (> 11 pigs) within 48 hours after farrowing.

Nursery experimental design

At weaning, pigs were transferred to the WCROC temperature-controlled nursery facility and were provided *ad libitum* access to feed and water during the 5-wk experiment. Each pen (2.4 × 1.2 m) consisted of plastic grated flooring, one cup drinker, and one 4-hole stainless steel self-feeder (Hog Slat® Inc., Newton Grove, NC). A total of 318 pigs were weaned from the 28 sows in previously described farrowing group, and 288 pigs were selected to be blocked according to BW and balanced by lactation dietary treatment across 36 pens. Each pen contained 8 pigs with no more than 2 littermates in each pen. Each block was assigned to one of four treatments (Lactation control – **LCon** and Nursery control – **NCon**; LCon and Nursery capsaicin – **NCap**; Lactation capsaicin – **LCap** and NCon; LCap and NCap) to provide 9 replications per treatment using a 2 × 2 factorial arrangement of treatments (Figure 2.1).

A 3-phase nursery feeding program was used, where phase 1 diets were fed from weaning to 7 d, phase 2 diets were fed from 8 to 21 d, and phase 3 diets were fed from 22 to 35 d post-weaning, with or without 1.0, 1.3, and 1.6 ppm capsaicin, respectively. All diets met or exceeded recommended NRC (2012) nutrient requirements using the NRC model for pig BW in each phase (Table 2.2). Individual pig body weight and pen feed disappearance were determined and recorded at each dietary phase change.

Growth performance data collection

All experimental pigs were weighed individually at weaning, and at the end of each diet phase for wks 1, 3 and 5 post-weaning. Pen feed disappearance was determined on the same day by subtracting the weight of any feed remaining in feeders from the total amount added to feeders during each respective phase period. Pen body weights and feed disappearance data from each phase were used to calculate ADG, ADFI and G:F for each phase and the overall 35-d feeding period. Pig mortalities were recorded to include the date, BW, and dietary treatment.

Blood sample collection

All pigs remained on their respective dietary treatments for an additional 3 d, and on d-38 post-weaning, one barrow closest to the pen mean BW (n = 36) in each pen was selected for collection of blood samples to determine if there were differences in gene expression among dietary treatments. Immediately after sampling, blood was transferred to another tube with an RNA shield solution. Each tube was labeled with piglet's ear tag number and stored at 4° Celsius.

RNA Extraction and gene expression

Total RNA from whole blood was extracted using a kit (Quick-RNA™ Whole Blood; Zymo Research, Irvine, CA) according to the manufacturer's instructions. The quantity of RNA quantity in each sample was measured by a Nano spectrometer (NanoPhotometer® N60, Implen, Westlake Village, CA). The RNA quality was assessed using gel electrophoresis by the QiAxcel Advanced System device (Qiagen®, Hilden, Germany), and 15 samples with the best RNA quality ratio (higher RNA Integrity Score) were selected and sent to the University of Minnesota

Genomics Center for analysis. Library prep was performed using a TakaraBio Clontech pico mammalian kit (SMARTer Stranded total RNA-seq Pico Input Mammalian kit, Takara Bio USA Inc., Mountain View, CA) to maximize quality reads. Reads were trimmed using FastQC and aligned to the pig genome, *Sus scrofa* v11.1, and differential gene expression was tested based on 2X absolute fold change cutoff and with False Discovery Rate (FDR) corrected *P*-values of < 0.05.

Statistical analysis

Nursery growth performance data were analyzed using SAS (v9.4; SAS Inst. Inc., Cary, NC). The model in the Mixed Procedure included the nursery dietary treatment nested within sow lactation treatment. The repeated statement of SAS was used with pen as experimental unit, and covariance structure was modeled using the autoregressive function. The final model was selected based on the Bayesian Criterion. All data were evaluated for outliers, normal distribution, and variances using the univariate procedure of SAS. The pen served as the experimental unit. Nursery BW coefficient of variation (CV %) was also calculated. Pair-wise comparisons among dietary treatment groups were used for determining significant differences in gene expression. Differences were considered when $P \leq 0.05$, and a trend when $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Lactation performance

Due to the limited number of sows (replications) per treatment ($n = 14$), there was not enough statistical power to accurately determine meaningful differences in sow and litter performance between control and capsaicin dietary treatments. Based

on Aaron and Hays (2004), assuming a coefficient of variation (CV) of 36,9% for the number of pigs weaned as a variable with a desired difference of at least 12% and with 80% of power and a significance level of 5%, the estimated number of sows per treatment is found to be approximately 134 sows for each treatment.

However, there was a trend ($P = 0.11$) for sows fed the capsaicin diet to consume less feed (6.92 kg/d) compared with sows fed the control diet (7.52 kg/d), which resulted in greater sow BW loss ($P < 0.05$) and backfat loss ($P < 0.05$) than sows fed the control diet. There were no differences in number of pigs born, born alive, stillborn, and weaned, nor were there differences in individual piglet and litter birth weight, weaning weight, and mortality between treatments. The optimal inclusion rate of capsaicin has not been determined in sow lactation diets. However, Ilsley (2003) and Matysiak (2012) found no effects on sow's performance with inclusions in feed of 2.2 ppm and 2 ppm of capsaicin respectively (as a part of a blend of plant extracts). Paraksa (2011) reported that inclusions in sow's gestation and lactation diets between 0 and 20 ppm capsaicin did not decrease feed intake. This may suggest that the inclusion of 2.5 ppm of capsaicin used in this experiment was within a safe inclusion range and that there may be an opportunity for testing greater inclusion levels.

Nursery growth performance

Low and variable feed intake after weaning commonly occurs in commercial pork production systems and is an important issue requiring solutions to achieve optimal health and growth performance of weaned pigs (Dong and Pluske, 2007). Holzer (1998) and Takeuchi et al. (1991) indicated that capsaicin-sensitive afferent neurons in stomach can be activated when capsaicin is consumed, and these neuronal

mechanisms play a central role in regulating various gastric functions such as secretion, mucosal blood flow, and motility, and also modulate the mucosal integrity. Furthermore, Zeng et al. (2019) showed that pigs exhibiting high ADG during the nursery period have greater likelihood of reaching full-market value compared to slow growing nursery. Therefore, we hypothesized that adding capsaicin to lactating sow and subsequent nursery diets may improve feed intake of weaned pigs during the critical first wk post-weaning.

There were no differences in pig BW at weaning and at the end of weeks 1, 3, and 5 among dietary treatments (Table 2.3). There were no differences in ADG or ADFI. However, there was a dietary treatment \times wk interaction ($P < 0.001$), where pigs from the LCap/NCap treatment had greater G:F (0.22), compared with pigs from LCon/NCon treatment (-0.46), with the LCon/NCap treatment (-0.11) and LCap/NCon (-0.03) treatments being intermediate. Under the conditions of this experiment is difficult to know if the difference in G:F is the product of greater ADG or reduced feed intake with similar unit of gain. It appears that both ADG and ADFI numerically increased, but future experiments are needed to undertint the nature of the improvement in G:F. Charal et al. (2016) reported that feeding anise to lactating sows increased the feed intake of nursery pigs.

There was a trend for greater ADFI ($P < 0.09$) and G:F ($P < 0.08$) responses based on nursery treatment nested within lactation treatment responses, suggesting that there appears to be a beneficial carry-over effect post-weaning for pigs that were nursing sows fed capsaicin on ADFI and G:F compared to those from sows fed the non-supplemented diet, and the effect is greater when pigs continued to be fed capsaicin diets after weaning. There are multiple mechanisms thereby

supplementation of lactation sow diets may increase feed intake of the offspring post-weaning. These include secretion of capsaicin and capsaicin metabolites in milk, excretion in feces, or through direct consumption of sow diets by pigs during the lactation period that allow newly weaned pigs to recognize the nursery pig diet as familiar.

The improvements in ADFI and G:F from capsaicin exposure did not result in an overall increase in BW at the end of the 35-d nursery feeding period. In fact, the CV in pig BW appeared to be greater at the end of wk 3 and wk 5 post-weaning for pigs exposed to capsaicin while nursing sows and/or pigs were consuming capsaicin during the nursery period compared with pigs with no capsaicin exposure from birth to 35-d post-weaning (Table 2.4).

Gene expression

The apparent differences in growth performance along with the interactive effects of time of capsaicin exposure, led us to evaluate potential differences in gene expression using blood samples collected from each of the four treatment combinations on d-38 of the nursery period. In comparison to the LCon/NCon treatment, nursery pigs from the capsaicin treated sows (LCap/NCon and LCap/NCap) had the most gene expression changes ($n = 6$; Table 2.5). Although the number of differentially expressed genes is small, these results indicate that either targeted changes occurred, or the biological effect was small.

The largest differentially expressed genes occurred in nursery offspring from sows that were fed capsaicin during lactation compared with sows and nursery pigs with no exposure to capsaicin. Differential gene expression was less in nursery pigs from sows fed the control diet regardless of whether they were fed capsaicin diets

during the nursery period. Upregulated and downregulated differential expression of genes is detailed in table 2.6. Genes FHDC1, REXO2 and MYH7 were downregulated when sows fed with capsaicin during lactation period and gene TXNRD3 was downregulated when pigs were fed with capsaicin after weaning. In this study only some undetermined genes were upregulated. These results suggest that feeding capsaicin to sows during lactation promotes similar gene expression changes as feeding capsaicin post-weaning when compared to control sows and nursery pigs. The overlap in differentially expressed genes between LCon/NCon and LCap and NCap pig groups indicates a shared pathway of induced genes by treatment, independent of treatment timepoint. The greater number of differentials expressed genes in the LCap group suggests a stronger lactational effect in nursery pigs than NCap.

In the present study several genes were detected in the analysis, but they have no previously described known function. Overall, capsaicin appeared to have a small, but consistent impact on gene expression when compared with pigs exposed to diets without capsaicin. Lui et al. (2014) obtained an expression differentiation of 490 genes in ileal mucosa of pigs fed 10 ppm of capsaicin in comparison with pigs fed the diet without capsaicin. However, we obtained only an expression of 12 genes but in whole blood samples of nursery pigs fed among 1 to 1.6 ppm of capsaicin versus pigs fed with nursery control diets.

In conclusion, the addition of capsaicin to diets of lactating sows and their weaned offspring appears to improve gain efficiency during the first wk post-weaning and may be a useful dietary intervention for overcoming low and variable feed intake which commonly occurs immediately after weaning. Although the number of genes

showing differential expression was small among dietary treatment combinations, these results may support that greater growth performance responses of nursery pigs during the first wk after weaning are achieved when they are weaned from sows fed capsaicin diets during lactation.

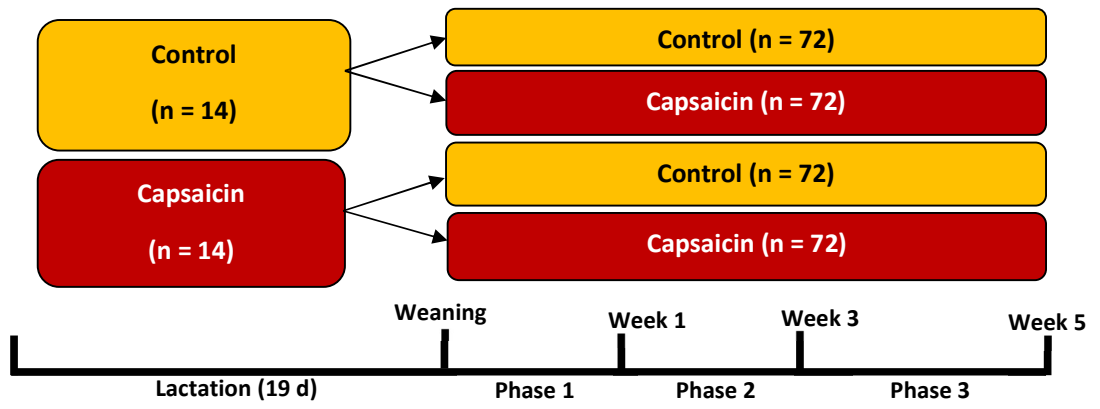


Figure 2.1. Experimental design and timeline.

Table 2.1. Ingredient and calculated nutrient composition of lactation diet¹

Item	Lactation
Ingredient Composition, %	
Corn	70.75
Soybean meal, 46.5% CP	24.25
Sow basemix ²	4.00
Soybean oil	1.00
Total	100.00
Calculated Nutrient Composition	
ME, kcal/kg	3320
DM, %	89.59
CP, %	17.15
Crude Fat, %	4.49
Ca, %	0.85
P, %	0.71
Standardized total tract digestible P, %	0.45
Na, %	0.22
Total Lys,%	0.90
Standardized ileal digestible	
Lys, %	0.78
Met, %	0.25
Met + Cis, %	0.52
Thr, %	0.55
Trp, %	0.17

¹Capsaicin at 2.5 ppm (125 ppm of Leader® Capsaicin 2%, Leader Bio-Technology Co., Ltd., Guangzhou, China) was added in the capsaicin treatment at the expense of soybean meal.

²Provided per kilogram of diet: 7.48 g of Calcium; 3.46 g of Phosphorus; 2.02 g of Sodium; 3.04 g of Chloride; 2,400,000 IU of vitamin A as retinyl acetate; 550,000 IU of vitamin D3; 18 IU of vitamin E as dl-alpha tocopheryl acetate; 1200.00 mg of vitamin K as menadione dimethylpyrimidinol bisulfite; 2400 mg of riboflavin; 9,000 mg of niacin; 6,000 mg of pantothenic acid as d-calcium pantothenate; 150,000 mg of choline as choline chloride; 6 mg of vitamin B12; 450 mg of folic acid; 60 mg of biotin; 300 mg of iodine as ethylenediamine dihydroiodide; 30 mg of selenium as sodium selenite; 15,000 mg of zinc as polysaccharide complexes; 16,500 mg of iron as ferrous sulfate; 1,500 mg of copper as copper sulfate; and 3200 mg of manganese as manganese oxide.

Table 2.2. Ingredient and calculated nutrient composition of nursery diets.

Item	Phase 1 ¹	Phase 2 ²	Phase 3 ³
Ingredient composition, %			
Corn	34.60	39.79	57.23
Soybean meal, 47.5% CP	25.00	28.01	35.96
Whey permeate	18.64	15.24	-
Whey protein concentrate	10.78	9.06	-
Lactose	4.72	2.50	-
Soybean oil	3.00	2.00	2.46
Monocalcium phosphate 21%	1.55	1.44	1.55
Limestone	0.89	1.10	1.30
Salt	0.19	0.30	0.45
Vitamin and trace mineral premix ⁴	0.50	0.50	0.50
L-lysine HCl	0.03	-	0.35
DL-methionine	0.10	0.06	0.11
L-threonine	-	-	0.09
Total	100.00	100.00	100.00
Calculated nutrient composition			
ME, kcal/kg	3557	3474	3369
DM, %	91.79	91.21	89.62
CP, %	24.20	24.48	22.27
Crude fat, %	6.00	5.00	5.00
ADF, %	2.32	2.63	3.55
NDF, %	5.56	6.32	8.70
Ca, %	0.86	0.90	0.89
P, %	0.76	0.74	0.74
Standardized total tract digestible P, %	0.48	0.45	0.39
Na, %	0.29	0.29	0.20
Total Lys,%	1.71	1.65	1.51
Standardized ileal digestible			
Lys, %	1.58	1.51	1.37
Met, %	0.48	0.45	0.43
Met + Cis, %	0.87	0.83	0.75
Thr, %	1.00	0.98	0.80
Trp, %	0.36	0.35	0.24

¹Phase 1 diets fed from weaning to day 7 post-weaning. Control diet contained no capsaicin and capsaicin diet contained 1 ppm of capsaicin (50 ppm of Leader® Capsaicin 2%) at the expense of soybean meal.

²Phase 2 diets fed from day 8 to day 21 post-weaning. Control diets contained no capsaicin and capsaicin diet contained 1.3 ppm of capsaicin (65 ppm of Leader® Capsaicin 2%) at the expense of soybean meal.

³Phase 3 diets fed from day 22 to day 35 post-weaning. Control diet contained no capsaicin and capsaicin diet contained 1.6 ppm of capsaicin (80 ppm of Leader® Capsaicin 2%) at the expense of soybean meal.

⁴ Provided per kilogram of diet: 2,200,000 IU of vitamin A as retinyl acetate; 550,000 IU of vitamin D₃; 17,6 IU of vitamin E as dl-alpha tocopheryl acetate; 880 mg of

vitamin K as menadione dimethylpyrimidinol bisulfite; 1,980 mg of riboflavin; 11,000 mg of niacin; 6,600 mg of pantothenic acid as d-calcium pantothenate; 99,000 mg of choline as choline chloride; 11 mg of vitamin B12; 440 mg of pyridoxine; 330 mg of folic acid; 220 mg of thiamine; 66 mg of biotin; 440 mg of iodine as ethylenediamine dihydroiodide; 59 mg of selenium as sodium selenite; 18,040 mg of zinc as zinc sulfate; 11,000 mg of iron as ferrous sulfate; 1,100 mg of copper as copper sulfate; and 3520 mg of manganese as manganese oxide.

Table 2.3. Effects of maternal and nursery capsaicin supplementation on nursery pigs¹ growth performance.

Item	LT ²	Control		Capsaicin		SEM	P-values		
	NT ³	Control	Capsaicin	Control	Capsaicin		NT(LT)	Wk	NT × Wk (LT)
Body Weight, Kg						0.46	0.51	<0.01	1.00
Week 0		6.51	6.51	6.63	6.64				
Week 1		6.35	6.49	6.63	6.83				
Week 3		10.04	10.28	10.28	10.58				
Week 5		20.19	20.66	20.35	21.01				
Average Daily Gain, g						17.81	0.38	<0.01	0.90
Week 1		-21.89	-1.78	-0.22	27.67				
Week 3		262.90	270.60	260.90	267.80				
Week 5		597.00	610.60	592.10	613.70				
Average Daily Feed Intake, g						17.43	0.09	<0.01	1.00
Week 1		66.67	76.00	82.89	95.44				
Week 3		305.30	334.30	323.10	343.00				
Week 5		812.70	844.20	838.40	854.90				
Gain: Feed						0.09	0.08	<0.01	<0.01
Week 1		-0.46	-0.11	-0.03	0.22				
Week 3		0.86	0.81	0.80	0.77				
Week 5		0.74	0.72	0.71	0.72				

¹A total of 288 weaned pigs distributed in 4 treatments

²Lactation treatment

³Nursery treatment

Table 2.4. Nursery body weight coefficient of variation (CV, %)

Time	LT ¹	CONTROL		CAPSAICIN	
	NT ²	CONTROL	CAPSAICIN	CONTROL	CAPSAICIN
Weaning		15.29	15.39	16.51	16.43
Week 1		13.52	13.04	16.03	15.71
Week 3		6.53	11.07	12.42	15.09
Week 5		4.26	10.33	10.28	12.79

¹Lactation diet treatment²Nursery diet treatment

Table 2.5. Comparison of whole blood gene expression among dietary treatment combinations in nursery pigs on d-38 post-weaning.

Dietary treatment combination comparison¹	No. genes	Gene names²
LCon/NCon vs. LCon/NCap	1	FHDC1
LCon/NCon vs. LCap/NCon	6	FHDC1, REXO2, 4 undescribed genes
LCon/NCon vs. LCap/NCap	6	FHDC1, REXO2, MYH7, MARCO, 2 undescribed genes
LCon/NCap vs. LCap/NCon	0	-
LCon/NCap vs. LCap/NCap	1	1 undescribed gene
LCap/NCon vs. LCap/NCap	3	TXNRD3, 2 undescribed genes

¹LCon = lactation control diet, LCap = lactation capsaicin diet, NCon = nursery control diet, NCap = nursery capsaicin diet

²Gene functions are as follows:

FHDC1 = FH2 Domain-Containing Protein 1, which is a microtubule-associated formin that regulates both actin and microtubule dynamics.

REXO2 = RNA Exonuclease 2 that encodes a 3'-to-5' exonuclease specific for small (primarily 5 nucleotides or less in length) single-stranded RNA and DNA oligomers.

MYH7 = Myosin Heavy Chain 7 that encodes the beta (or slow) heavy chain subunit of cardiac myosin.

MARCO = Macrophage Receptor with Collagenous Structure encodes a protein that is a member of the class A scavenger receptor family and is part of the innate antimicrobial immune system.

TXNRD3 = Thioredoxin Reductase 3 is a selenocysteine-containing flavoenzyme which reduce thioredoxins, as well as other substrates, and plays a key role in redox homeostasis.

Table 2.6. Tagwise dispersion - fold change between treatments¹.

Genes*	Genes' interactions ¹					
	A	B	C	D	E	F
FHDC1 ²	-8.24	-5.59	-5.95			
REXO2 ³		-9.12	-9.53			
MYH7 ⁴			-67.84			
MARCO ⁵			-29.57			
TXNRD3 ⁶						-3.36
Und. 1 ⁷		-44.93	-21.45			
Und. 2 ⁸		-25.31				15.60
Und. 3 ⁹		-20.31				
Und. 4 ¹⁰		-10.72				
Und. 5 ¹¹			-39.37			
Und. 6 ¹²					11.66	
Und. 7 ¹³						4.75

*Positive values (+) are upregulated genes and negatives values (-) are downregulated genes

¹Genes' interaction:

A: LCon/NCon vs. LCon/NCap

B: LCon/NCon vs. LCap/NCon

C: LCon/NCon vs. LCap/NCap

D: LCon/NCap vs. LCap/NCon

E: LCon/NCap vs. LCap/NCap

F: LCap/NCon vs. LCap/NCap

²FHDC1 = FH2 Domain-Containing Protein 1

³REXO2 = RNA Exonuclease 2

⁴MYH7 = Myosin Heavy Chain 7

⁵MARCO = Macrophage Receptor with Collagenous

⁶TXNRD3 = Thioredoxin Reductase 3

⁷Undetermined gene, feature ID: ENSSSCG00000010190

⁸Undetermined gene, feature ID: ENSSSCG00000038275

⁹Undetermined gene, feature ID: ENSSSCG00000050649

¹⁰Undetermined gene, feature ID: ENSSSCG00000035293

¹¹Undetermined gene, feature ID: ENSSSCG00000041816

¹²Undetermined gene, feature ID: ENSSSCG00000035226

¹³Undetermined gene, feature ID: ENSSSCG00000030882

Chapter 3 – Effects of gut microbiota modifying feed additives on growth performance of nursery pigs

SUMMARY

The objective of this study was to determine the effect of gut microbiota modifying feed additives on the growth performance of nursery pigs weaned from primiparous dams compared with pigs weaned from multiparous sows. A total of 1,000 newly weaned pigs from 21 primiparous female litters and 75 multiparous sow litters were selected and assigned to 50 pens in a randomized complete block design. Four dietary treatments included pharmacological levels of zinc (**Zn**) and copper (**Cu**) in phases 1 and 2 and consisted of 1) control diets (**CON**), 2) CON with 0.5% of *Aspergillus* fermentation extract (**ASP+**), 3) CON with 0.05% of a mixture of *Bacillus* strains (**BC**), and 4) CON with 0.1% of *Lactobacillus* fermentation products (**LFP**) were fed along with an additional treatment consisting of 5) ASP+ but without pharmacological levels of Zn and Cu (**ASP-ZnCu**). Phase 3 consisted in the same 5 treatments but without pharmacological levels of Zn and Cu in all cases. All pigs were fed a common diet for phase 4. Individual pig body weight (**BW**) was recorded at weaning (d-21) and at the end of nursery (63 d of age), while pen weight and feed disappearance were recorded weekly. Injections of medications and fecal scores were also recorded, and morbidity was calculated based on the number of pigs that were removed and placed in non-test pens. Pen-based data were analyzed using a generalized linear mixed model with average BW at weaning serving as the random effect and dietary treatment as the fixed effect. Time was used as a repeated measure with pen as the repeated unit. Initial BW was used as

covariable. Individual pig initial and final BW data were analyzed using a mixed model with the effect of parity and the interaction of sow parity with dietary treatments. Morbidity data were analyzed using a binomial distribution. Number of pigs receiving injections of medications and fecal scores were analyzed with Chi-Square. Pigs from multiparous sows had greater BW at weaning and on d-42 post-weaning than pigs from gilt litters ($P < 0.01$). There were no interactions between dietary treatments and dam parity on final weight, weight gain, average daily gain (**ADG**) and morbidity. Individual BW gain was greater for pigs fed CON compared with those fed ASP+ diets ($P < 0.01$). Morbidity was greater in pigs weaned from primiparous females compared with those from multiparous sows ($P < 0.01$), but there were no differences in morbidity among dietary treatments or interactions between dam parity and dietary treatments. Pen weight gain and feed intake in ASP-ZnCu was the least ($P < 0.01$) among treatments for the first 4-weeks post-weaning, but there were no differences among the other dietary treatments. Four weeks after weaning, BW gain and average daily feed intake (**ADFI**) were not different among dietary treatments. Pigs fed diets with gut microbiota modifying feed additives had less loose or liquid feces compared with CON ($P < 0.05$), with the LFP treatment providing the best fecal scores of any of the other dietary treatments. In conclusion, gut microbiota modifying feed additives did not enhance growth performance of nursery pigs but may support better fecal scores.

Key words: *Aspergillus* fermentation extract, *Bacillus* strains, copper, growth performance, *Lactobacillus* fermentation product, nursery pigs, zinc.

INTRODUCTION

Direct fed microbials (DFM) and prebiotics may provide growth promoting benefits when added to the diets of nursery pigs (Wenk 2000; Choct 2009; Cheng et al. 2014). The microbiome appears to play a crucial role in pig health and feeding prebiotics and DFM has been shown to increase the population of beneficial bacteria in the gastrointestinal tract, which stimulates the production of lactic and acetic acid, leading a reduction of luminal pH and increased fermentation with the production of SCFA as a result. (Liu et al., 2018). However, the effectiveness among these feed additives may vary and depend on pig age, body weight, or duration of feeding. Indeed, of 92 studies summarized, the addition of prebiotics to pig diets improved ADG, ADFI, or gain efficiency in only 11% of these studies (Schweer et al., 2017). The relatively low effectiveness of these feed additives may be due to multiple factors such as disease prevalence, weaning age, and maternal immune and microbial ecology.

Progeny of primiparous dams have reduced growth performance and higher morbidity compared to progeny from multiparous sows, which has been attributed to the less mature gastrointestinal (**GI**) tract of primiparous female-derived progeny than multiparous sow progeny (Wijesiriwardana et al., 2019). The underdevelopment of the GI tract includes less effective barrier function (Miller et al. 2012) and a different microbial ecology (Carney-Hinkle, 2013). Bacterial genera are passed from sows to piglets which affects the microbiome of pigs during its productive life. Furthermore, microbiome from piglets have less microbial diversity than in sows in the gastrointestinal tract and vagina (Tran et al., 2019). Considering this, adding gut microbiota modifying feed additives such

as DFM and prebiotics to nursery diets, may improve the growth performance of pigs weaned from primiparous sows compared with those weaned from multiparous sows. Therefore, the aim of this study was to evaluate the impact on the growth performance of nursery pigs fed gut microbiota modifying additives weaned from primiparous dams compared with pigs weaned from multiparous sows in a commercial nursery feeding program. We hypothesized that feeding diets containing gut microbiota modifying additives to nursery pigs would enhance growth performance of nursery pigs and reduce variability in BW among pigs derived from primiparous and multiparous sows.

MATERIALS AND METHODS

Facilities and animal management

This study was conducted at the Freking sow farm facilities of New Fashion Pork Inc. (NFP) in Jackson, MN, and the Koster Research Nursery facility located in Round Lake, MN. Progeny from 96 crossbred sows obtained from litters of 21 primiparous and 75 multiparous females were selected to provide the 1,000 weaned pigs (PIC® TR4 x (Fast LW x PIC® L02, Hendersonville, TN)) for use in this study.

Experimental design

At weaning, pigs were moved and housed in an environmentally controlled nursery facility. Each pen (4.8 × 2.4 m) consisted of plastic grated flooring, one cup drinker, and one 4-hole stainless steel self-feeder (Hog Slat® Inc., Newton Grove, NC). Access to feed and water was provided *ad libitum*. All selected pigs were differentiated

according to the dam's parity using tags with different colors and assigned to 50 pens to achieve a similar BW (with a standard deviation of 0.08 from an average weight of 5.75 kg) and sex distribution. Each pen contained 20 pigs with at least 1 pig from a primiparous dam in each pen. Pens were randomly assigned to one of the five dietary treatments consisting of corn and soybean meal-based diets in a 4-phase feeding program where phases 1 to 3 were budgeted to provide an established amount of each diet to be consumed (Table 3.1), and a common non treatment for Phase 4 diet were offered ad libitum until day 42 post-weaning. All diets were formulated to meet or exceed NRC (2012) model nutrient requirement specifications (Table 3.2). Gut microbiota feed additives were included in diets during phases 1, 2, and 3 (Table 3.3), and pharmacological dietary concentrations of zinc (2,880 ppm of Zn from ZnO) and copper (232 ppm of Cu from tribasic CuCl₂) were added in 4 of the 5 dietary treatments in phase 1 and 2 diets. Dietary treatments consisted of 1) control diets (CON), 2) 0.5% of *Aspergillus* fermentation extract (ASP+), 3) 0.05% of a mix of *Bacillus* strains (BC), 4) 0.1% of *Lactobacillus* fermentation products (LFP), and 5) 0.5% of *Aspergillus* fermentation extract and without Zn and Cu at pharmacological levels (ASP-ZnCu).

Growth performance data collection

All pigs were weighed individually at weaning, and at the end of nursery period (42-d post-weaning). Individual BW data were used to calculate overall BW gain and ADG for each dietary treatment and the interaction between primiparous female-derived and multiparous sow-derived pigs.

Pen weight was determined and recorded weekly along with pen feed disappearance which was determined by subtracting the weight of the feeders and the remaining feed inside from the total amount added to feeders during the preceding week. Pen BW and feed disappearance data from each phase were used to calculate ADG, ADFI, and gain efficiency (**G:F**) for each phase and the overall 42-d feeding period. For pens where pigs were removed for poor health, ADFI was calculated based on the remaining number of pigs in each pen after pig removals which occurred on the same day of feed weight backs.

Husbandry, monitoring health status, and medications

Pigs were observed once daily for signs of poor health. Individual injectable medications were administered when pigs displayed signs of being lethargic, had poor locomotion, or severe diarrhea. Pigs showing weakness or continual poor growth after medications were removed to non-test pens and included in morbidity calculations. Date, weight and dietary treatment of pigs removed from the experiment were recorded. There were no mortalities during this study. Number of pigs receiving injections of medications and fecal scores were recorded, and a fecal scoring system based on feces consistency being: solid = 1, soft = 2, and liquid = 3. Fecal scores were observed only during the first week after weaning, every day and individually. Every nonsolid feces observation was registered as one event with the corresponding score.

Statistical analysis

This study was designed as a one-way treatment structure with pen serving as the experimental unit. Pens of pigs were allotted to treatment in a randomized complete block

design. Pen-based observations of ADG, ADFI, and G:F were analyzed using a generalized linear mixed model coded on PROC MIXED procedures of SAS (SAS Institute, Cary, NC), with average BW at weaning serving as the random effect and dietary treatment as the fixed effect. Time was used as repeated measures with pen as the repeated unit, while initial BW was used as covariable. Data are reported as the least square means, and means were separated by multiple contrasts of least squared differences.

Data from individual pig observations (initial and final BW) were analyzed using a generalized linear mixed model similar to that previously described, but it also contained the effect of parity and the interaction of sow parity with dietary treatments. Morbidity data were based on total number of pigs removed from pens for each dietary treatment were analyzed using a binomial distribution in PROC GLIMMIX of SAS, and data are reported as the odds ratios of likelihood of events. Number of injections and fecal score data were analyzed using Chi-Square test for independence. Outliers, normal distributions, and variances were evaluated on the residuals after model fitness using graphs of quantiles, boxplots, and dispersion of residuals. Differences were considered when $P \leq 0.05$, and a trend when $0.05 < P \leq 0.10$.

RESULTS

Individual pig growth performance

There were no differences in BW at weaning among dietary treatments and, as expected, based on Smits and Collins (2009) findings, piglets derived from primiparous

dams had lower BW ($P < 0.01$) than piglets from multiparous sows (Table 3.4). Pigs fed CON diets had the greatest ($P < 0.01$) final BW, followed by pigs fed BC and LFP. Pigs fed ASP+ and ASP-ZnCu had the lightest final body weight, being ASP+ the lowest ($P < 0.01$). There was no interaction between dietary treatments and parity of dam on the individual pig initial BW or final BW indicating that the dietary treatment did not affect the growth performance of pigs from primiparous dams differently than pigs from multiparous sows. Individual pig BW gain and ADG were greatest ($P < 0.01$) among pigs fed CON diets and least for pigs fed ASP+ diets. Removal of sick pigs (morbidity) was greater ($P < 0.01$) for pigs weaned from primiparous females compared with those from multiparous dams. Nonetheless, there were no differences among dietary treatments or interactions of dam parity and dietary treatments. However, the difference in the removal rate between primiparous sow-derived pigs fed ASP-ZnCu (14.63%) and ASP+ (12.77%) treatments was greater ($P < 0.01$) than the removal rate of primiparous dam-derived pigs fed CON diets (8.70%).

Pen-based growth performance

For pen-based growth performance analysis, average pen weight and feed intake of each pen was calculated weekly (Table 3.5). The BW of pigs fed ASP-ZnCu was the lowest for the first 4 wk (treatment \times week interaction $P < 0.01$), but there were no differences in BW among the other treatments. However, after the first 4 wk, the BW of pigs fed ASP-ZnCu was not different from those fed any other treatment. Similarly, ADG of pigs in ASP-ZnCu was least for the first 3 wk post-weaning (treatment \times week interaction; $P < 0.01$), but there were no differences in the ADG among treatments at the

end of the 6 wk experiment. For the first 4 wk post-weaning, the ADFI of pigs fed ASP-ZnCu was less (interaction $P > 0.01$) than pigs fed CON. However, during the last 2 wk post-weaning, ADFI of pigs fed ASP-ZnCu was not different from pigs fed the other dietary treatments. There were no differences in ADFI among treatments for the overall period.

Pigs not responding to injectable medications or requiring additional interventions were removed from test pens into non-test pens for the remainder of the study. There were no statistical differences among treatments (Table 3.8). Therefore, weight and feed consumption by pen were measured weekly during the 6 wk nursery period (Table 3.6). Consistent with individual pig BW and the pen average BW observations, the ADG of pigs in pens fed CON was greater ($P < 0.01$) than pigs fed ASP-ZnCu. The daily pen weight gain between treatments was different for the first 3 wk post-weaning (treatment \times week interaction; $P < 0.01$) but not during the last 3 wk post-weaning. During wk1 post-weaning, pigs fed ASP-ZnCu lost BW, while pigs fed all other treatments had small increments in weight gain. The slow ADG in pigs fed ASP-ZnCu was the result of less ($P < 0.01$) ADFI than pigs fed any of the other dietary treatments during the first 4 wk of the experiment (treatment \times week interaction; $P < 0.01$). Despite some recovery in feed intake of pigs in pens fed ASP-ZnCu, the overall feed intake was the lowest among treatments ($P < 0.01$).

Variability in BW of groups of pigs at the end of the nursery is associated with variation in market weight (López-Vergé et al., 2018). Therefore, we investigated if dietary treatments decreased variability in BW by the end of the nursery period. Body

weight variability was analyzed by calculating and comparing the coefficient of variation (CV) at weaning and at the end of the nursery period among dietary treatments (Table 3.7). At weaning were no differences in BW variation among treatments ($P = 0.47$). However, at the end of the 6 wk nursery period, there were differences ($P < 0.05$) in the BW CV among dietary treatments, where the greatest CV was for pigs fed LFP diets compared with those fed ASP-ZnCu diets, which had the lowest CV of all dietary treatments. As said before, this lower variability for ASP-ZnCu treatment would be beneficial for market weight. However, ASP-ZnCu had more antibiotic shots and loose stools events than the other treatments in this study.

Health and fecal scores

There were no differences among dietary treatments for the number of pigs receiving antibiotic treatments. With that we may conclude that there no treatment effect on pigs' health. Furthermore, removed animals which were considered as morbidity does not showed differences as well (Table 3.8). However, there were differences in fecal scores among dietary treatments ($P = 0.04$), where pens of pigs fed LFP diets presented fewer ($n = 4$) soft or liquid stool events than pigs fed ASP+ and BC which presented 10 and 12 soft or liquid stool events, respectively. Pens fed CON and ASP-ZnCu had the greatest incidence of liquid or soft stools events ($n = 17$ each). No mortalities were registered in this study.

DISCUSSION

Several feed additives have been developed to support the post-weaning growth performance of young pigs by improving the immune response and by regulating the

development of post-weaning microbiota (Lui et al., 2018). However, effectiveness of these feed additives is low (Schweer et al., 2017). Consequently, it is necessary to understand the management, environmental, and animal health conditions that modify the gastrointestinal microbial communities and their function. It is also unknown if the microbiota composition of primiparous dam-derived progeny will benefit more from use of DFM or prebiotics than progeny from multiparous sows.

Diets containing DFM or prebiotics did not improve the nursery pig growth performance in this experiment. Lower BW gain and ADFI during the first 3 and 4 weeks after weaning, were observed for pigs consuming the diet with non-pharmacological doses of Zn and Cu compared with the other treatments which was expected since high dietary levels of Cu and Zn can increase the growth performance of newly weaned pigs (Jacela et al., 2010). These pigs were able to attain similar BW at the end of the experiment. This similar BW appeared to be the result of pigs fed ASP-ZnCu consuming a marginally greater amount of feed and improved gain to feed ratio at the end of the nursery period. In general pigs showed good health conditions during this study since there were no mortality, only a few liquid feces events and no evidence of respiratory issues. Mortality ratio for U.S. farms between 2012 and 2017 was 4.62% (Stalder, 2018). Therefore, we speculate that pigs in this experiment were able to express some compensatory growth during subsequent weeks post-weaning due to the relatively good health conditions. Zeng et al. (2019) supports these observations because they reported that pigs with faster ADG during nursery have greater overall ADG compared to slow growing nursery pigs at a similar birth weight.

There are multiple possible reasons for the lack of effect of dietary treatments on the growth performance of pigs in this experiment. The high health status of this group of pigs may be one of the reasons why the feed additives used in this experiment did not cause significant effects on growth performance. High health animals are more able to express their growth potential, with this there would not be enough room for additive to enhance growth pigs' performance. Growth performance used to be similar when health status is similar, performance would be influenced by diet complexity (Dritz et al., 1996). Another reason that may have limited the growth promotion effect of the feed additives evaluated in this study is that nursery diets in this experiment contained many additives that may have complementary or antagonist interactions with these products. In a study conducted by Højberg et al. (2005) with a pharmacological level of Zn in newly weaned pigs' diets (2500 ppm of Zn from ZnO), a decrease of lactic acid bacteria and lactobacilli growth was observed compared with animals receiving nutritional levels of Zn (100 ppm of Zn from ZnO). Furthermore, the inclusion of pharmacological levels of zinc oxide is commonly recommended to avoid post-weaning diarrhea (Poulsen, 1998; Smith et al., 1997; Hill et al., 2000; Hu et al., 2012) and also a combination of organic acids containing medium-chain fatty acids have been demonstrated to reduce pathogenic activity (Zentek et al., 2013). Gulhane et al. (2018) observed that different copper sulphate solutions may inhibit *Lactobacillus* spp. However, results of several studies with gut microbiota modifying feed additives have been inconsistent because each additive are diet and health status dependent (Lui et al., 2018).

In conclusion, under the conditions of this experiment, we reject the first hypothesis that gut microbiota modifying products will enhance post-weaning growth

performance of pigs in addition to feeding diets pharmacological doses of zinc and copper. However, we would say that gut microbiota modifying products inclusion on nursery feed may support better stools quality which suppose less medicinal treatments and better nutrient absorption through healthier enterocytes. It is unknown if addition of gut microbiota modifying products to diets without pharmacological doses of zinc and copper would have a different effect. Therefore, we recommend that this study would be repeated with diets without pharmacological doses of zinc and copper.

Table 3.1. Feeding scheme.

Item	Phase 1	Phase 2	Phase 3	Phase 4
Budget (Kg/pig) *	2.42	3.63	3.63	<i>ad libitum</i> **

*Feed phases offered until established budget was consumed

**Phase 4 were offered *ad libitum* until day 42 of nursery period

Table 3.2. Ingredient and calculated nutrient composition of experimental diets.

Item	Phase 1	Phase 2	Phase 3	Phase 4
Ingredient composition				
Ground corn (particle size 600 μ)	43.27	45.46	42.00	34.38
Soybean meal, 47.5% Crude Protein	18.75	22.50	28.65	28.50
Corn gluten meal, 60% Crude Protein	7.50	6.75	0	0
Cereal blend ¹	5.80	2.80	1.40	0
Distillers dried grains with solubles	5.00	10.00	17.50	30.00
Oat groats	5.00	0	0	0
Poultry meal	5.00	5.00	3.75	0
Vegetable oil blend	3.50	3.00	3.40	0
Beef tallow	0	0	0	3.55
Spray dried whey	1.25	0	0	0
Calcium carbonate	0.95	0.80	1.00	1.28
Mono-calcium phosphate, 21%	0.65	0.60	0.38	0.50
Lysine HCL, 98/78.8%	0.64	0.66	0.52	0.52
Zinc oxide 72%	0.40	0.40	0	0
Copper chloride ²	0.04	0.04	0	0
Acidifier ³	0.50	0.50	0	0
Salt	0.49	0.49	0.50	0.58
Nursery vitamin and trace mineral premix ⁴	0.25	0.25	0.25	0
Grower vitamin and trace mineral premix ⁵	0	0	0	0.15
Flavor ⁶	0.25	0.10	0	0
Threonine	0.19	0.21	0.19	0.13
L-Methionine	0.11	0.14	0.29	0.21
L- Tryptophan	0.11	0.11	0.09	0.05
Vitamin E	0.10	0	0	0
Medium chain fatty acids ⁷	0.10	0.05	0	0
L-Valine	0.09	0.10	0.10	0
Choline chloride, 60 %	0.03	0.03	0	0
Alpha galactosidase ⁸	0.03	0.01	0	0
Phytase, 2500 FTU ⁹	0	0	0	0.03
Total	100.00	100.00	100.00	100.00
Calculated nutrient composition				
Dry Matter, %	74.14	79.84	86.41	82.82
Metabolizable energy, kcal/kg	3,369	3,308	3,333	3,289
Net energy, kcal/kg	2,589	2,523	2,522	2,426
Crude protein, %	21.21	23.64	23.30	23.77
Crude fat, %	6.80	6.30	6.75	6.72
Acid detergent fiber, %	3.10	3.61	4.63	5.93
Neutral detergent fiber, %	6.69	7.94	9.88	12.58
Total calcium, %	0.78	0.70	0.68	0.69

Total phosphorus %	0.60	0.62	0.61	0.65
Total Lysine %	1.44	1.56	1.57	1.55
SID Lys, %	1.30	1.40	1.40	1.36
Standardized ileal digestible amino acid ratios				
Met + Cys/Lys	0.62	0.62	0.62	0.60
Thr/Lys	0.63	0.63	0.63	0.61
Trp/Lys	0.22	0.22	0.22	0.20
Val/Lys	0.69	0.69	0.69	0.66
Iso/Lys	0.56	0.56	0.56	0.59
Leu/Lys	1.45	1.42	1.18	1.31
His/Lys	0.34	0.35	0.35	0.37
Sodium, %	0.35	0.35	0.35	0.35
Copper, ppm	248.81	248.81	16.81	162.00
Magnesium, ppm	153.20	129.05	161.25	205.56
Selenium, ppm	0.30	0.30	0.30	0.30
Zinc, ppm	3008	3008	120	100

¹ Bakery by products.

²Tri-basic copper chloride; IntelliBond C; Micronutrients, Indianapolis, IN.

³Encapsulated organic acids; AcidoMatrix; Novus Int., St. Louis, MO.

⁴Provided per kilogram of diet: 10,400 IU vitamin A, 447 IU vitamin D3, 1,341 IU vitamin D, 70.1 IU vitamin E, 0.04 mg vitamin B₁₂, 8.1 mg riboflavin, 41.38 mg niacin, 27.5 mg pantothenic acid, 1.70 mg folic acid, 2.1 mg thiamine, 4.00 mg pyridoxine, 0.40 mg biotin, 250.00 mg vitamin C, 3.7 mg menadione. Also provided trace minerals per kilogram of diet: 18.5 mg Cu from tribasic Cu, 1.2 mg I from Ca iodate, 221.5 mg Fe from ferrous sulfate, 44 mg Mn from tribasic Mn, 44 mg organic Mn, 0.33 mg Se from sodium selenite, 0.33 mg organic Se, 132 mg tribasic Zn, 132 mg organic Zn.

⁵Provided per kilogram of diet: 5,520 IU vitamin A, 250.0 IU vitamin D3, 17.6 mcg vitamin D, 26.4 IU vitamin E, 21.5 mcg vitamin B₁₂, 4.5 mg riboflavin, 25.5 mg niacin, 16.4 mg pantothenic acid, 2.3 mg menadione. Also provided trace minerals per kilogram of diet: 33 mg Cu from mixed organic and inorganic sources, 0.9 mg I from Ca iodate, 165.2 mg Fe from ferrous sulfate, 49.6 mg Mn from mixed organic and inorganic sources, 0.70 mg Se from sodium selenite, 221 mg Zn from mixed organic and inorganic sources.

⁶Flavor blend; N-Hance; Kindstrom-Schmoll, Inc. Eden Prairie, MN.

⁷Lauric acid; LipoVital GL90; Berg + Schmidt, Libertyville, IL.

⁸Alpha-galactosidase; AlphaGal280 P, Kerry Agribusiness, Charleville, Cork, Ireland.

⁹*Escherichia coli* derived 6-phytase, Origination Inc., Maplewood, MN.

Table 3.3. Description of dietary treatment and products.

Treatment	Additive	Doses	Description
CON	Control ¹	-	Corn and soybean meal-based diet program
ASP-ZnCu	Amaferm®*	0.50%	<i>Aspergillus oryzae</i> fermentation extract
ASP+	Amaferm® ²	0.50%	<i>Aspergillus oryzae</i> fermentation extract
BC	Comfortfeeds™ ³	0.05%	<i>Bacillus subtilis</i> , <i>B. amyloliquefaciens</i> , <i>B. licheniformis</i>
LFP	Syngex® ⁴	0.10%	<i>Lactobacillus acidophilus</i> fermentation product

¹New Fashion Pork, Inc, Jackson, Minnesota, USA.

²BioZyme, Inc, St. Joseph, Missouri, USA.

³Bioverse, Inc., Worthington, Minnesota, USA.

⁴Diamond V, Cedar Rapids, IA, USA.

*All treatments except treatment ASP-ZnCu contained pharmacological doses of Zn (2880 ppm of Zn from ZnO) and Cu (232 ppm of Cu from tribasic copper chloride), on phases 1 and 2.

Table 3.4. Individual pig-based weaning body weight (WBW), final body weight (FBW), body weight gain (gain), average daily gain (ADG), and morbidity of gilt or sow derived nursery pigs¹ fed diets containing gut microbiota modifying products.

Item	WBW, kg	FBW, kg	Gain, kg	ADG, g	Morbidity, %
Treatment					
CON ²	5.77	19.62 ^a	13.80 ^a	329 ^a	5.53
ASP-ZnCu ³	5.80	18.82 ^{bc}	12.99 ^{bc}	309 ^{bc}	6.74
ASP ⁺ ⁴	5.80	18.46 ^c	12.64 ^c	301 ^c	9.05
BC ⁵	5.72	19.26 ^{ab}	13.44 ^{ab}	320 ^{ab}	8.70
LFP ⁶	5.65	19.12 ^{ab}	13.29 ^{ab}	317 ^{ab}	6.00
SEM	0.10	0.24	0.24	0.01	0.33
<i>P</i> -values	0.90	0.01	0.01	0.01	0.35
Parity					
Gilt progeny	5.59 ^b	18.46 ^b	12.63 ^b	301 ^b	10.81 ^a
Sow progeny	5.89 ^a	19.66 ^a	13.83 ^a	329 ^a	4.25 ^b
SEM	0.06	0.15	0.15	< 0.01	0.21
<i>P</i> -values	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Treatment x parity					
CON × gilt	5.76	19.12 ^{cd}	13.32 ^{cd}	317 ^{cd}	8.70
CON × sow	5.78	20.10 ^a	14.27 ^a	340 ^a	4.64
ASP-ZnCu × gilt	5.69	18.25 ^{de}	12.43 ^{de}	296 ^{de}	14.63
ASP-ZnCu × sow	5.89	19.38 ^{bc}	13.56 ^{bc}	323 ^{bc}	7.59
ASP+ × gilt	5.50	17.73 ^e	11.91 ^e	284 ^e	12.77
ASP+ × sow	6.02	19.19 ^{cd}	13.37 ^{cd}	318 ^{cd}	3.97
BC × gilt	5.64	18.79 ^{cde}	12.96 ^{cde}	307 ^{cde}	9.76
BC × sow	5.80	19.74 ^{abc}	13.92 ^{abc}	331 ^{abc}	2.56
LFP × gilt	5.35	18.38 ^{de}	12.55 ^{de}	299 ^{de}	8.70
LFP × sow	5.95	19.86 ^{ab}	14.03 ^{ab}	334 ^{ab}	2.61
SEM	0.14	0.33	0.33	< 0.01	0.46
<i>P</i> -values	0.21	0.89	0.89	0.88	0.64

¹A total of 1,000 weaned pigs PIC TR4 × (Fast LW × PIC L02); PIC®, Hendersonville, TN, USA; Fast Genetics Inc, Saskatoon, SK, Canada.

²Positive control based on corn and soybean meal. Phases 1 and 2 contained pharmaceutical levels of Cu and Zn.

³Negative control diet containing prebiotic (Amaferm®, Biozyme Inc., St Joseph, MO) without pharmacological levels of copper and zinc in phases 1 and 2.

⁴As positive control with additional prebiotic (Amaferm®, Biozyme Inc., St Joseph, MO).

⁵As positive control with additional direct fed microbial (Comfortfeeds™ Bioverse Inc., Worthington, MN).

⁶As positive control with additional *Lactobacillus acidophilus* fermentation product (Syngen X®; Diamond V Inc., Cedar Rapids, IA).

^{a-c}Means within a column indicate differences among dietary treatments, differences between sow progeny, or interaction of dietary treatment and sow progeny type differ ($P < 0.05$).

Table 3.5. Weekly growth performance of the average nursery pigs¹ fed diets containing gut microbiota modifying products.

Item	Treatments ²⁻⁶					SEM	<i>P-value</i> ⁷		
	CON	ASP-ZnCu	ASP+	BC	LFP		T	W	T*W
BW, kg						0.08	<0.01	<0.01	<0.01
Week 1	5.58 ^{ab}	5.34 ^b	5.66 ^a	5.63 ^a	5.59 ^{ab}				
Week 2	6.94 ^{ab}	6.32 ^c	7.12 ^a	6.90 ^{ab}	6.66 ^{bc}				
Week 3	9.40 ^a	8.42 ^b	9.26 ^a	9.13 ^a	9.31 ^a				
Week 4	12.28 ^a	11.24 ^b	11.86 ^{ab}	11.89 ^{ab}	12.14 ^a				
Week 5	14.92	14.25	14.35	14.42	14.66				
Week 6	19.79	19.17	19.05	19.45	19.53				
ADG, g						0.01	0.29	<0.01	<0.01
Week 1	10.9 ^{ab}	-7.5 ^b	30.9 ^a	29.4 ^a	19.6 ^a				
Week 2	193.8 ^{ab}	139.7 ^c	208.1 ^a	181.9 ^{abc}	152.6 ^{bc}				
Week 3	351.5 ^{ab}	299.0 ^b	306.8 ^b	318.5 ^{ab}	378.2 ^a				
Week 4	411.1	403.1	370.9	394.7	404.5				
Week 5	376.8	429.8	355.6	360.4	359.2				
Week 6	695.7	703.1	671.9	719.2	696.0				
Overall	340.0	327.8	324.1	333.9	334.8				
ADFI, g.						0.03	<0.01	<0.01	<0.01
Week 1	19.3	16.6	24.9	26.7	28.3				
Week 2	162.2 ^a	129.9 ^b	170.5 ^a	161.8 ^a	163.1 ^a				
Week 3	353.0 ^a	293.9 ^b	366.4 ^a	357.3 ^a	345.0 ^a				
Week 4	540.6 ^a	446.0 ^b	486.6 ^{ab}	497.5 ^{ab}	545.7 ^a				
Week 5	602.0	583.5	564.7	606.6	597.9				
Week 6	892.8	909.0	856.4	905.5	894.7				
Overall	428.2	396.4	411.6	425.9	429.1				
G:F						0.01	0.06	<0.01	<0.01
Week 1	0.145 ^{ab}	-0.151 ^b	0.430 ^a	0.416 ^a	0.256 ^a				
Week 2	0.938 ^{ab}	0.802 ^{bc}	0.966 ^a	0.88 ^{ab}	0.735 ^c				
Week 3	0.885 ^{ab}	0.884 ^{ab}	0.746 ^c	0.794 ^{bc}	0.973 ^a				
Week 4	0.701	0.834	0.699	0.730	0.691				
Week 5	0.582	0.684	0.579	0.548	0.557				
Week 6	0.742	0.741	0.746	0.757	0.742				
Overall	0.720	0.744	0.711	0.711	0.709				

¹A total of 1,000 weaned pigs PIC TR4 × (Fast LW × PIC L02); PIC, Hendersonville, TN, USA; Fast Genetics, Saskatoon, SK, Canada] allocated in 50 pens for 10 pens per dietary treatment.

²Positive control based on corn and soybean meal. Phases 1 and 2 contained pharmaceutical levels of Cu and Zn.

³Negative control diet containing prebiotic (Amaferm, Biozyme, St Joseph, MO) without pharmacological levels of copper and zinc in phases 1 and 2.

⁴As positive control with additional prebiotic (Amaferm, Biozyme, St Joseph, MO).

⁵As positive control with additional direct fed microbial (Comfortfeed^{sTM}, Bioverse, Worthington, MN).

⁶As positive control with additional *Lactobacillus acidophilus* fermentation product (Syngen X; Diamond V Inc. Cedar Rapids, IA).

⁷Main effect of treatment (T) and week post weaning (W), and interaction of dietary treatment and week (T x W).

^{a-c}Means within a row indicate differences among dietary treatments for the respective week of the experiment differ ($P < 0.05$).

Table 3.6. Weekly growth performance (kg) of the pens of nursery pigs¹ fed diets containing gut microbiota modifying products.

Items	Treatments ²⁻⁶					<i>P</i> -value ⁷			
	CON	ASP-ZnCu	ASP+	BC	LFP	SE M	T	W	T*W
Pen weight						6.55	< 0.01	< 0.01	< 0.01
Weaning	19.90	20.25	20.38	20.33	20.97				
Week 1	21.44 ^{ab}	19.21 ^b	24.69 ^a	24.46 ^a	23.74 ^{ab}				
Week 2	42.92 ^a	30.56 ^b	48.76 ^a	47.16 ^a	42.58 ^a				
Week 3	88.91 ^a	66.25 ^b	88.35 ^a	89.93 ^a	92.88 ^a				
Week 4	141.16 ^a	116.55 ^b	135.56 ^a	142.54 ^a	145.55 ^a				
Week 5	190.69	169.48	180.82	188.40	194.22				
Week 6	282.40	258.74	269.45	285.15	288.19				
Daily pen gain						0.11	< 0.01	< 0.01	< 0.01
Week 1	0.22 ^{ab}	-0.15 ^b	0.62 ^a	0.59 ^a	0.39 ^{ab}				
Week 2	3.69 ^{ab}	2.61 ^c	4.00 ^a	3.57 ^{ab}	3.01 ^{bc}				
Week 3	6.68 ^{ab}	5.45 ^b	5.86 ^b	6.21 ^{ab}	7.34 ^a				
Week 4	7.70	7.30	7.01	7.65	7.78				
Week 5	7.08	7.73	6.64	6.88	6.95				
Week 6	13.10	12.75	12.66	13.82	13.43				
Overall	6.41	5.95	6.13	6.45	6.48				
Daily pen feed intake						0.65	< 0.01	< 0.01	< 0.01
Week 1	0.32	0.27	0.43	0.47	0.51				
Week 2	3.00 ^a	2.30 ^b	3.19 ^a	3.09 ^a	3.14 ^a				
Week 3	6.59 ^a	5.23 ^b	6.89 ^a	6.88 ^a	6.60 ^a				
Week 4	10.03 ^a	7.97 ^b	9.09 ^{ab}	9.56 ^a	10.43 ^a				
Week 5	11.19	10.40	10.47	11.54	11.45				
Week 6	16.67	16.28	15.99	17.28	17.17				
Overall	7.97 ^{ab}	7.08 ^b	7.68 ^{ab}	8.14 ^a	8.22 ^a				
Pen gain						1.20	< 0.01	< 0.01	< 0.01
Week 1	1.54 ^{ab}	-1.04 ^b	4.31 ^a	4.13 ^a	2.77 ^{ab}				
Week 2	21.47 ^a	11.35 ^b	24.06 ^a	22.70 ^a	18.84 ^{ab}				
Week 3	45.99 ^{ab}	35.68 ^c	39.59 ^{bc}	42.77 ^{abc}	50.30 ^a				
Week 4	52.26	50.30	47.22	52.62	52.66				
Week 5	49.53	52.94	45.26	45.85	48.67				
Week 6	91.71	89.26	88.62	96.75	93.98				
Overall	262.50	238.49	249.06	264.82	267.22				
Pen intake						4.50	< 0.01	< 0.01	< 0.01

Week 1	3.54	3.16	4.32	4.61	4.85
Week 2	22.29 ^a	17.42 ^b	23.62 ^a	22.91 ^a	23.29 ^a
Week 3	47.44 ^a	37.94 ^b	49.541 ^a	49.47 ^a	47.48 ^a
Week 4	71.50 ^a	57.078 ^b	64.91 ^{ab}	68.20 ^{ab}	74.27 ^a
Week 5	79.67	74.14	74.61	82.05	81.50
Week 6	118.03	115.28	113.24	122.27	121.52
Overall	369.05 ^a	331.42 ^b	356.74 ^{ab}	375.93 ^a	379.50 ^a

¹A total of 1,000 weaned pigs PIC TR4 × (Fast LW × PIC L02); PIC, Hendersonville, TN, USA; Fast Genetics, Saskatoon, SK, Canada] allocated in 50 pens for 10 pens per dietary treatment.

²Positive control based on corn and soybean meal. Phases 1 and 2 contained pharmaceutical levels of Cu and Zn.

³Negative control diet containing prebiotic (Amaferm, Biozyme, St Joseph, MO) without pharmacological levels of copper and zinc in phases 1 and 2.

⁴As positive control with additional prebiotic (Amaferm, Biozyme, St Joseph, MO).

⁵As positive control with additional direct fed microbial (Comfortfeed^{sTM}, Bioverse, Worthington, MN).

⁶As positive control with additional *Lactobacillus acidophilus* fermentation product (Syngen X; Diamond V Inc. Cedar Rapids, IA).

⁷Main effect of treatment (T) and week post weaning (W), and interaction of dietary treatment and week (T x W).

^{a-c}Means within a row indicate differences among dietary treatments for the respective week of the experiment differ ($P < 0.05$).

Table 3.7. Effects of gut microbiota modifying feed additives on variability of nursery pigs¹.

Item	Treatments ²⁻⁶					SEM	P-value
	CON	ASP-ZnCu	ASP+	BC	LFP		
Initial BW, CV	21.72%	19.65%	20.29%	20.36%	20.94%	0.008	0.47
Final BW, CV	18.66% ^{ab}	18.28% ^a	19.36% ^{ab}	20.49% ^{ab}	21.31% ^b	0.008	0.05

¹A total of 1,000 weaned pigs PIC TR4 × (Fast LW × PIC L02); PIC, Hendersonville, TN, USA; Fast Genetics, Saskatoon, SK, Canada].

²Positive control based on corn and soybean meal. Phases 1 and 2 contained pharmaceutical levels of Cu and Zn.

³Negative control diet containing prebiotic (Amaferm®, Biozyme Inc., St Joseph, MO) without pharmacological levels of copper and zinc in phases 1 and 2.

⁴As positive control with additional prebiotic (Amaferm®, Biozyme Inc., St Joseph, MO).

⁵As positive control with additional direct fed microbial (Comfortfeeds™ Bioverse Inc., Worthington, MN).

⁶As positive control with additional *Lactobacillus acidophilus* fermentation product (Syngen X®; Diamond V Inc., Cedar Rapids, IA).

Table 3.8. Effects of gut microbiota modifying products on health-related events of nursery pigs¹.

Item	Treatments ²⁻⁶					P-value
	CON	ASP-ZnCu	ASP+	BC	LFP	
Removed animals, % ⁷	5.53	6.74	9.05	8.70	6.00	0.35
Treatments ⁸	98	121	97	99	102	0.43
Loose stools events ⁹	17	17	10	12	4	0.04

¹A total of 1,000 weaned pigs PIC TR4 × (Fast LW × PIC L02); PIC, Hendersonville, TN, USA; Fast Genetics, Saskatoon, SK, Canada].

²Positive control based on corn and soybean meal. Phases 1 and 2 contained pharmaceutical levels of Cu and Zn.

³Negative control diet containing prebiotic (Amaferm®, Biozyme Inc., St Joseph, MO) without pharmacological levels of copper and zinc in phases 1 and 2.

⁴As positive control with additional prebiotic (Amaferm®, Biozyme Inc., St Joseph, MO).

⁵As positive control with additional direct fed microbial (Comfortfeeds™ Bioverse Inc., Worthington, MN).

⁶As positive control with additional *Lactobacillus acidophilus* fermentation product (Syngen X®; Diamond V Inc., Cedar Rapids, IA).

⁷Percentage of removed pigs with morbidity signs from approximately 200 pigs by treatment.

⁸Total of antibiotic shots by treatment.

⁹Total of loose stools events during first week after weaning by treatment, the scores of fecal softness were as follow (1 = normal feces, 2 = loose stools, and 3 = liquid and watery diarrhea).

Overall Summary

Agricultural production of food will need to increase by 70 percent (nearly 100 percent in developing countries) by 2050 to cope with a 40 percent increase in world population (Robinson et al, 2011). This increasing demand for food, and particularly animal protein, has led to a more intensive farming practices for swine and poultry. In addition to improvements in management techniques, genetics, and nutrition, antibiotics have been used extensively during the last decades to improve growth performance by modifying gut microbiota and improving the health status of the animals (Dibner and Richards 2005). However, several countries have restricted or banned the use of antibiotics because of several public health issues involving bacterial resistance to antibiotics. Therefore, several feed additives like essential oils, plant extracts, and gut microbiota modifying products may be possible solutions to improve animal health and growth performance in the absence of using antibiotics because of their capability to enhance digestive enzyme secretions and nutrient absorption, reduction of gut pathogens, antioxidant properties, and immune status improvement (Zeng et al., 2015). Nonetheless, the effectiveness among these products is dependent on the health status and production conditions of pigs because they vary among production systems (Langendijk et al., 2007; Liu et al., 2018).

The research included in this thesis involves evaluating several types of growth promoting feed additives and feeding strategies. First, we tested the hypothesis that feeding a feed additive to the lactating dam will improve weaning transition of the offspring. Therefore, we tested the pre-weaning exposure of pigs to capsaicin while

nursing sows, and subsequent growth performance and gene expression of pigs post-weaning fed diets containing capsaicin (Chapter 2). In a second study, the effect of feeding newly weaned pigs diets containing gut microbiota modifying feed additives on growth performance were evaluated and compared among pigs derived from primiparous dams and those from multiparous sows (Chapter 3).

Results discussed in Chapter 2 suggested that adding capsaicin to diets of lactating sows and their offspring after weaning may have a positive effect on gain efficiency during the first week after weaning, due to overcoming low and variable feed intake which often occurs to newly weaned pigs. It is unknown if the level of capsaicin included in feed, impacted gain efficiency because of flavor or some other mechanism in piglets' development such as enhanced digestion capacity or capsaicin anti-inflammatory properties at GI tract level. Furthermore, there were only a few but consistent number similar genes showing differential expression among dietary treatments. The greatest changes in expressed genes were observed in pigs from sows fed capsaicin. New experiments are needed using higher capsaicin doses for nursery pigs' and in lactation diets of sows. It may also be beneficial to study if the benefits of capsaicin in lactation diets is greater in gilts than multiparous sows. Taking samples for RNA sequencing from blood and some other tissues at weaning and at the end of nursery period would be needed in order to understand the impact of feeding diets containing capsaicin on gene expression, the functions of these genes, and their relationship with growth performance of pigs coming from gilts and from adult sows.

Results from the study described in Chapter 3 suggested that the inclusion of gut microbiota modifying products to diets containing pharmacological doses of zinc and copper and fed to newly weaned pigs did not enhance post-weaning growth performance regardless of whether pigs were derived from primiparous or multiparous sows. Additional research is needed to determine if gut microbiota modifying products would have a different effect when added to diets without pharmacological doses of zinc and copper. A better understanding of the role of microbiota diversity and composition of piglets derived from primiparous sows and its relationship with their growth performance and health status is also needed.

In conclusion, capsaicin and gut microbiota modifying feed additives have not shown significant positive effects on overall growth performance of nursery pigs when added to their diets and/or their mothers' diets. However, additional studies should be conducted to evaluate whether improvements in growth performance is observed under conditions of greater health challenges, diet composition and additive inclusion rates.

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