ABSTRACT

SEVAROLLI LOFTUS, ANA LUCIA. Effects of Dietary Inclusion of Direct-Fed Microbial on Growth Performance and Carcass Traits of Growing-Finishing Pigs. (Under the direction of Dr. Sung Woo Kim).

The research hypothesis is that dietary supplementation of mono-strain and multi-strain DFM may possibly enhance feed intake, nutrient uptake, muscle deposition, and consequently growth performance and carcass traits of growing-finishing pigs.

The first study (Chapter 2) used 60 pigs based on a randomized complete block design to evaluate the effects of mono-strain DFM, *Lactobacillus acidophilus* ($1x10^9$ cfu/g), on the growth performance and carcass traits of finishing pigs. The inclusion levels of mono-strain DFM were 0.28, 0.24, and 0.20% during phase 5, 6, and 7, respectively. In current study, the dietary supplementation of mono-strain DFM did not affect ($P > 0.10$) growth performance and loin quality, but tended to decreased ($P = 0.088$) backfat thickness from the first rib and decreased ($P < 0.05$) backfat thickness from the last rib.

The second study (Chapter 3) used 60 pigs based on a randomized complete block design to evaluate the effects of multi-strain DFM, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* ($1x10^8$ cfu/g) on growth performance and carcass traits of growing-finishing pigs. The inclusion levels of multi-strain DFM were 0.05% during phase 6 and 7. The dietary supplementation of multi-strain DFM increased feed intake ($P < 0.05$), carcass yield ($P < 0.05$), and tended to increase ($P = 0.062$) growth of growing-finishing pigs, however, loin quality was not affected ($P > 0.10$).

Effects of Dietary Inclusion of Direct-Fed Microbial on Growth Performance and Carcass Traits of Growing-Finishing Pigs

by
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DEDICATION

To my family, Jeffrey Michael Loftus, Elenira da Silva Sevarolli, Dorival Sidney Sevarolli, Mario Sergio Sevarolli, Marcia Regina Sevarolli, Helen Evans, Billy Evans, and Matthew Loftus. Their love, kindness, and support helped me through my graduate program.

BIOGRAPHY

Ana Lucia Sevarolli Loftus, daughter of Dorival Sidney Sevarolli and Elenira Sevarolli was born in Sao Paulo, Brazil. Ana received her major in Veterinary Medicine in 2010 at Sao Paulo State University in Jaboticabal, Sao Paulo, Brazil. In 2011, Ana was accepted at University of Florida, Gainesville, Florida. She obtained a Master of Agribusiness degree in 2012. From 2012 to 2013, Ana worked in the swine industry as a Supply Chain Specialist and obtained experience in swine production, grain procurement, and quality assurance in the United States and South America. In 2013, Ana had the opportunity to continue her education and was accepted in the Department of Animal Science of North Carolina State University under the direction of Dr. Sung Woo Kim.

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LITERATURE REVIEW

Introduction

World meat production has quadrupled from 78 million tons to 311.6 million metric tons from 1960 to 2015 being pork the most widely eaten meat representing 37% of human protein intake (FAO, 2015). In 2013, 113 million metric tons of pork were consumed worldwide, especially by China and the European Union (FAOSTAT, 2014). China accounts for 53 million metric tons of global pork consumption and this trend seems to rise due to rapidly growing of Chinese economic and urbanization, whereas the European Union accounts for 22.4 million metric tons of global pork consumption (FAOSTAT, 2014).

Versatility, price, nutritional value, and food safety led pork to be the most consumed meat around the globe (McCarthy et al., 2004). Pork is fully consumed in multiple ways, fresh, processed, and cured. Pork by-products, such as pig's jowl, feet, and skin are also extensively used in many cultural traditional dishes (Ockerman and Basu, 2014).

Since the late 90's and early 2000's, retail pork price declined and consumption increased. The swine industry became more efficient in production cost through genetic improvements, technology innovation, greater production capacity, and improved packing line system. Cuts are leaner, lower in calories and cholesterol than poultry and beef (Patterson et al., 2009).

However, as any other livestock production, the swine industry has been facing challenges that may negatively affect the producers. The new antimicrobial regulation aims to ban the administration of antimicrobials as growth-promoters (FDA, 2013), which is a widespread production practice to increase growth rate, improve feed conversion, and prevent disease outbreaks (FDA, 2014). The indiscriminate use of antimicrobials has been

associated to the development of antimicrobial resistance (AMR) rising concerns in meat consumers (Marshall and Levy, 2011).

The additional challenge is the use restriction or ban of growth-promoters by the main pork importers. Ractopamine is one of the most widely used component for swine growth-promoters, commonly added to finisher feeds, but FDA is currently reviewing its regulation (Centner et al., 2014). Ractopamine seems to enhance protein synthesis and decrease protein degradation (Bergen et al., 1987; Merkel et al., 1987). The enhancement of growth performance, nitrogen retention, leanness, and carcass yield are the overall effects of this drug on pigs (Anderson et al., 1987a; Veenhuizen et al., 1987).

Many livestock producers in the United States are seeking alternatives to the use of antimicrobials and growth-promoters once the new regulation may considerably affect the livestock industry and generate negative consequences to the United States meat export market. A current alternative strategy is the dietary inclusion of direct-fed microbial (DFM), which might enhance animal's intestinal microbiota balance, boost immunity, improve growth performance, and enhance carcass quality (Krehbiel et al., 2014).

The aim of this chapter is to review scientific evidence of the use of DFM, and to discuss the possibility of using DFM as an alternative to the new antimicrobial regulations and use restriction of growth-promoters in swine industry by measuring the effects of DFM on the overall health, growth performance, and carcass traits of growing-finishing pigs.

Probiotics

History of Probiotics

Since ancient times, humans have consumed probiotic microorganisms through fermented foods. Fermentation is a traditional preservation technique, which improves food taste, texture, and color (Chelule et al., 2010). Besides adding sensory attributes to the food, fermenting microorganisms are considered health-promoter due to their positive effects on the gut microbiota and overall health status of the host animals. Early studies suggested that *Lactobacillus* and *Bifidobacterium* species inhibit pathogenic microbial activity, decrease putrefaction, and thus, prevent gut infection (Metchnikoff, 1907).

The term probiotic was probably first used in 1954 by Vergio when he described "Probiotika" as a beneficial substance for gut microorganisms (Vergio, 1954). In 1989, Fuller defined probiotic as "a live microbial feed supplement, which beneficially affects the host animal by improving gut microbial balance". In 1992, Havenaar altered the definition including the benefits of probiotic over respiratory and urogenital tract, and its potential benefit on animal's growth performance. Once probiotic has a broad definition, the U.S. Food and Drug Administration (FDA) and the Association of American Feed Control Officials (AAFCO) required the use of DFM nomenclature instead of probiotic, when viable bacteria or yeast are present in animal feeds (AAFCO, 1999; FDA, 2003; FDA, 2014).

The use of DFM as livestock feed additive has increased sharply since the 1970's due to potential benefits on animals' productivity (Fuller, 1992). In the past, DFM showed favorable effects on growth performance of rodents (Hargrove and Alford 1980; Wong et al., 1983). In lactating cows, dietary supplementation of DFM increased milk yield and

decreased incidences of acidosis (Piva et al., 1993). Beef cattle fed DFM had enhanced fiber digestion and gut health (Elam et al., 2003; Newbold et al., 1996). In pigs, DFM showed benefits on growth performance and feed intake of piglets (Mathews et al., 1998) and growing-finishing pigs (Pollmann et al., 1980). Other studies showed immunity enhancement (Qiu et al., 2012) and absence of drug resistance (Rastall, 2004) when animals were fed DFM.

Besides the potential benefits of DFM, several questions regarding the mechanisms of action are still not fully answered. Also, benefits are not always consistent, stimulating scientists to explain these variations through extensively studies.

Direct-Fed Microbial

Not all the feed or foods containing live microorganisms would be considered DFM. A desired DFM must not be pathogenic; survive the unfavorable gut environment to be able to produce benefits; express optimal gut adherence; be stable and viable during manufacturing and storage process; and enhance intestinal microbiota balance, growth performance, and immune system (Fuller, 1989; Pal, 1999) (Table 1).

Table 1. Desirable characteristics of DFM microorganisms

Strain properties	**Technological functions**
Acid and bile stability	Survive the GIT environment and be viable after manufacturing process
Adherence to the gut	Competitive pathogens' exclusion of pathogens
Colonization of the gut	Proliferation in the gastrointestinal tract
Antimicrobial production	Release of volatile acid and bacteriocins
Safety	Accurate strain identification.
Effectiveness validation	Dose-response determination.

Source: Adapted from Salminen et al., 1996

Direct-fed microbial is commonly available as mono-strain, multi-strain, and multi-specie. Research studies encourage the usage of multi-strain or multi-specie mixtures due to better gut colonization rate and efficacy (Timmerman et al., 2004; Guo et al., 2010). The major strains approved by the Food and Drug Administration and the Association of American Feed Control Officials (FDA and AAFCO, 1998) are in Table 2.

Table 2. Microorganisms approved for animal feeds

Aspergillus sp.	*B. capillosus*	*L. brevis*	*L. reuterii*
A. niger	*B. ruminocola*	*L. buchneri*	***Leuconostoc sp.***
A. oryzae	*B. suis*	*L. bulgaricus*	*L. mesenteroides*
Bacillus sp.	***Bifidobacterium sp.***	*L. casei*	***Pediococcus sp.***
A.coagulans	*B.adolescentis*	*L. cellobiosus*	*P. cerevisae*
B. lentus	*B. animalis*	*L. curvatus*	*P. pentosaceus*
B. licheniformis	*B. infantis*	*L. delbruekii*	***Saccharomyces sp.***
B. pumilus	*B. longum*	*L. ferciminis*	*S. cerevisae*
B. subtilis	*B. thermophilum*	*L. fermentum*	***Enterococcus sp.***
Bacteroides sp.	*B. bifidum*	*L. helveticus*	*E. cremoris*
B. amylophilus	***Lactobacillus spp.***	*L. lactis*	*E. diacetylactis*
B. amylophilus	*L. acidophilus*	*L. plantarum*	*E. faecium*

Source: Adapted from Muirhead, 2001.

Major Microorganisms Used as DFM in Swine Production

Direct-fed microbial is based on live microorganisms that enhance animal's health condition and performance. The main microorganisms present in the DFM formulas are *Lactobacillus, Enterococcus,* and *Bifidobacterium*. The gastrointestinal tract is their natural habitat making them able to survive the unfavorable gut environment conditions (Chang,

2001; Simpson, 2004) and produce antimicrobial substances, which prevent pathogenic bacteria adhesion and disease outbreaks (Lee et al., 2009).

Lactobacillus spp.

Lactobacillus spp. are a gram-positive, non-spore forming and facultative anaerobic bacteria naturally present in the mucosa of the small intestinal of human and animals, soil, plants, manure, and spoilage (Hammes and Vogel, 1995). Mostly known as strictly fermentative, lactobacilli mainly produce lactic acid, carbon dioxide, and acetic acid after degradation of carbohydrates, proteins, and vitamins (Saarela, 2009).

Lactobacilli are also the main bacteria in pigs' microbiota often present in the small intestine (Franklin et al., 2002; Konstantinov et al., 2006) and the distribution depends on the pH, substrate specificity, mucus presence, and microorganism's interactions. More than 80 lactobacilli species have been discovered, but *Lactobacillus acidophilus* is one of the most common strains present in probiotic's compositions (Gomes and Malcata, 1999).

Several studies have been performed to quantify the efficacy of *Lactobacillus acidophilus* in growing-finishing pigs. However, there was a lack of consistency among the results. In 1986, Pollmann found no relationship between *Lactobacillus acidophilus* and growth performance. On the other hand, Fuller (1989) expressed a positive relationship between *Lactobacillus* and pigs' growth performance. In 1991, Cromwell conducted multiple studies using *Lactobacillus acidophilus*, which some increased pig's weight gain and feed intake, whereas in others no effect on growth performance was detected.

Enterococcus spp.

Enterococcus is a gram-positive lactic acid bacterium present in the gastrointestinal tract of human and animals, and extensively used in food fermentation and as DFM (Franz et al., 1999). In pigs, *Enterococcus faecium* showed the ability to survive at low pH environment demonstrating constant amounts of 1.8 x 10^8 cfu/g in sow feces (Macha et al., 2004). In piglets, *Enterococcus faecium* may decrease the incidence of *E. coli* diarrhea (Underdahl et al., 1982; Taras et al., 2006) and prevent infectious after birth by the enhancement of immune modulation (Pollmann et al., 2005).

Bifidobacterium spp.

Bifidobacterium is a gram-positive, non-spore forming, non-motile, and obligate anaerobic bacteria, commonly present in the large intestine (Sgorbati et al., 1995). More than 30 species of *Bifidobacterium* were isolated from human and animal gastrointestinal tract (Gomes and Malcata, 1999*).* Bifidobacteria have mono-, di- and oligosaccharides as main substrates, which provide them a competitive advantage (Biavatti et al., 2000). Studies have shown that Bifidobacteria persisted in the gut for up to six weeks after the DFM ingestion has ended (O'Connell, 2009).

Recently, Bifidobacteria have been widely applied in human nutrition. Live strains have become a new ingredient for conventional yogurt recipes due to potential health benefits against infantile diarrhea (Saavedra et al., 1994; Chouraqui et al., 2004; Orrhage et al., 1994), lactose intolerance (Jiang et al., 1996), colorectal cancer (Pool-Zobel et al., 1996), constipation (Kleesen et al., 1997; Kumemura et al., 1992), and LDL cholesterol (Xiao et al., 2003).

The use of *Bifidobacterium* strains in human nutrition runs parallel to similar developments in animal feed industry. *Bifidobacterium spp.* have been commercialized as direct-fed microbial for ruminants and non-ruminants. In pigs, *Bifidobacterium thermophilum* has been extensively applied as a dietary supplement to combat *Salmonella* infection. However, the impacts on growth performance are not consistent (Tanner et al., 2014).

Direct-fed microbial: Mechanisms of Action

Swine and any other livestock are submitted to high stress environment, which challenge the animals' homeostasis, growth performance, and meat quality. The first barrier against potential disease factors is the gastrointestinal tract. The dietary inclusion of Direct-fed microbial enhances intestinal microbiota balance, which inhibits the growth of pathogenic bacteria, stimulate the immune response, and eventually improve weight gain and carcass yield. The DFM mode of action has not been clearly elucidated, but some hypotheses were described by Fuller (1989) and Roff (1991) and will be explained below.

Development of Unfavorable Environment for Pathogens

The gut environment is crucial for bacteria survival. Antimicrobials and growth-promoter drugs normally disrupt gut defense by altering the pH and microbiota, which beneficiates pathogenic invasion (Edens, 2003). Contrary, direct-fed microbial seems to create an unfavorable environment for pathogenic bacteria proliferation. Direct-fed microbial microorganisms inhibit the adherence, replication, and action of enteropathogens through the production of volatile acids, lactic acid, carbon dioxide, hydrogen peroxide, and bacteriocins. According to Powell (1996), antimicrobial peptides act in several different ways (Table 3).

Volatile acids

Lactic acid bacteria release volatile acids in the small and large intestine upon carbohydrate digestion. Volatile acids decrease intestinal pH creating a hostile environment for harmful bacteria. In addition, these substances have the ability to diffuse bacteria cellular membrane and alter vital metabolic functions. This is one of the reasons that direct-fed microbial may protect animals and human from bacterial infections.

Carbon Dioxide and Hydrogen Peroxide

Carbon dioxide and hydrogen peroxide are lactic acid bacteria fermentation by-products. The carbon dioxide produces an anaerobic environment making aerobic bacteria survival and colonization unfeasible, whereas hydroxyl radicals ($^{-}$OH) from hydrogen peroxide destroys bacteria nucleic acids and cell proteins.

Bacteriocins

The lactic acid bacteria fermentation produces bactericidal and bacteriostatic substances. Some bacteriocins bind cytoplasm receptors preventing enteric colonization, while others bind membrane receptors forming membrane pores generating pathogens' death.

Table 3. Antimicrobial products produced by DFM and its inhibitory effect

Antimicrobial	Effect
Volatile Fatty Acids	Decrease gut pH and alters pathogen's metabolism
Carbon dioxide	Create an anaerobic environment unfeasible for some pathogens
Hydrogen peroxide	Interacts with free radicals destroying pathogens' cellular membrane
Bacteriocins	Occupy enteric receptors or form pores on pathogens cell membrane

Source: Adapted from Mishra and Lambert, 1996.

Pathogen Inhibition

The single epithelium cell layer separates the small and large intestinal content from the bloodstream, however, the intestinal segments have different morphology and absorptive functions. The small intestine structure is based on crypts and villi that maximize nutrient absorption, whereas the large intestine is a uniform organ with minimal absorptive functions. However, both have binding sites that mediate non-pathogenic and pathogenic bacteria signaling (Wells et al., 2010). This signaling cascade is well known as a cross-talk mechanism, which translate indigenous and pathogen bacteria message to the host. The cross-talk mechanism combines three events: Attachment and invasion, modification of intestinal barrier function, and inflammation (Koehler et al., 2003). According to Fuller (1975), the bacterial polysaccharide membrane attaches to the host intestinal epithelium blocking the receptors. Recent studies have suggested that bacteria present in direct-fed microbial compete for intestinal binding sites preventing pathogen's adhesion and cross-talk cascade events (Figure 1) (Reid, 2001).

Besides binding site competition, bacteria from direct-fed microbial are also capable to inhibit pathogenic adhesion by stimulating mucus secretion. Gastrointestinal tract is covered by two intestinal mucus layers. The inner portion is a sterile and thinner layer that facilitate nutrient absorption (Atuma et al., 2001), whereas the outer layer is not sterile, rich in glycoproteins, non-specific antimicrobial molecules, and immunoglobulin (McGuckin et al., 2011). Both represent a physical barrier against undesirable bacteria invasion. Elucidative studies have described the stimulatory effect of direct-fed microbial over intestinal mucus synthesis (Mack et al., 1999; McGuckin et al., 2011). Deplancke and Gaskins (2001) found

abundant amount of goblet cells and thicker mucus layer in conventionally raised animals than germ-free ones, suggesting that indigenous microbes play an important rule on mucin secretion. Although this stimulatory effect has not been clarified yet, it indicates that direct-fed microbials stimulate goblet cells through bioactive factors (Deplancke and Gaskins, 2001). Therefore, the combination of receptor competition and mucus secretion may prevent bacterial infection without necessarily kill the bacteria, which is a desirable way to avoid the development of selective pressure.

Stimulation of Immune Response

In order to maintain animal homeostasis, innate or acquired immune systems stimulate systemic and mucosal immune responses. In mammals, mucosal immune system is the primary defense against pathogenic bacteria, viruses, and parasites (Galdeano and Perdigon, 2006). After cross-talk to intestinal mucosa, DFM microorganisms release soluble factors, which trigger immune response through the stimulation of specialized lymphoid aggregates (GALT), adenoids, Peyer's patches, and single lymphoid nodules (Blaise et al., 2007).

The primary signs of immune stimulation consist on the propagation of lymphocytes, cytokines, antibodies, enhancement of natural killer cell cytotoxicity, and macrophage activity (Kato et al., 1983, Famularo et al., 1997; Wu, 2006). In contrast with the pathogen inhibition mechanism, direct-fed microbial may stimulate the immune system even after microbe's death. The remaining genetic material may activate T-helper cells, which kill intracellular parasites and disseminate autoimmune responses (Klinman et al., 1996). More

details about the association of DFM microorganisms and immune modulation are still unknown, but evidences have supported it not merely in animals but in human as well.

Studies using weaned piglets have investigated the relationship between direct-fed microbial and immunity during enteric infections. The weaning period is critical and intestinal disorders are likely to occur due to lack of maternal antibodies and the microbiota adjustment to a new diet inclusion. Superior immune response followed by low level of pathogenic bacteria invasion were found on piglets fed lactic acid bacteria, especially *Lactobacillus spp*. and *Bifidobacterium spp.* (Fox, 1988; Shu et al., 2001; Lee et al., 2012; Yang et al., 2014).

Other studies showed that germ-free animals had immature intestinal immune system before the insertion of commensal microbes (Blaise et al., 2007). Oral doses of *Lactobacillus acidophilus* in germ-free animals increased antibodies and leukocyte levels (Pollmann et al., 1980). *In vitro* tests also reaffirmed that non-pathogenic bacteria may influence mucosal immune response through the secretion of immunomodulins (Hemaiswarya et al., 2013).

Although numerous studies have linked direct-fed microbial with immunity enhancement, there is insufficient information about the mechanism of action. Current researches about microbe's gene expression aim to clarify certain uncertainties and amplify the use of direct-fed microbial as a prophylactic alternative against critical diseases.

Direct-Fed Microbial: Impacts on Growth Performance

Animal food production is marked by large-scale with an average of thousands animals per herd. In the United States, swine, poultry, beef, and dairy commodities have ad-libitum access to water and they are fed a well-balanced diet combined with low doses of

antimicrobials and growth-promoters to accelerate growth and compensate for unsanitary conditions (FDA, 2013). These drugs decrease the nutrient competition in the gut and constrain the release of toxic metabolites from pathogenic bacteria, which influence negatively animals' weight gain (Dibner and Richards, 2005). Antimicrobials, such as tetracycline and carbadox are claimed to enhance anabolic reactions and, consequently, increase lean muscle deposition (Harsh et al., 1964; Moser et al., 1980).

The widely use of low doses of antimicrobials for longer period has been reviewed. The antimicrobial ban by Europe Union and other countries can generate negative impacts in the U.S. meat export market, which may strengthen this sensitive topic nationally (Maron et al., 2013).

Animal producers are seeking alternatives to maintain productivity levels without the use of restricted drugs resurging the interest in probiotics. The modulation of immunity, inhibition of pathogenic bacteria, release of digestive enzymes and vitamins, and improvement of performance and meat quality are some benefits associated with the dietary supplementation of direct-fed microbial (Fernandes et al., 1987; Ross et al., 2009). Direct fed-microbial also seemed to increase villi height and width of small intestine, which are essential components for nutrient absorption being an important regulator of weight gain and feed efficiency (Suo et al., 2012).

Although, the direct-fed microbial mode of action is still unknown and the groeth performance benefits are not always consistent, some studies had suggested the benefits to include direct-fed microbial in basal diets. According to Pollman et al. (1980), nursery pigs fed basal diet combined with a commercial *Lactobacillus acidophilus* probiotic increased

ADG by 4.5% and feed conversion by 7.2%. Choi et al. (2014) showed an increase of phase II pigs growth performance fed corn-soybean meal diet with 0.60% of *Lactobacillus acidophilus* ($1x10^2$ cfu/g), *Bacillus subtilis* ($2x10^4$ cfu/g), *Saccharomyces cerevisae* ($1.2x10^2$ cfu/g) and *Aspergillus oryzae* ($1x10^3$ cfu/g).

Giang et al. (2011) showed that growing-finishing pigs fed a mixture of *Lactobacillus acidophilus* ($1x10^7$ cfu/g), *Saccharomyces cerevisae* ($4.3x10^6$ cfu/g) and *Bacillus subtilis* ($2x10^6$ cfu/g) significantly improved feed intake and feed efficiency. A second study including *Bacillus subtilis* ($1x10^{10}$ cfu/mL) and *Clostridium butyricum* ($1x10^9$ cfu/g) increased pigs' average daily gain and feed efficiency.

On the other hand, Shon et al. (2005) did not observe growth enhancement of growing-finishing pigs fed *Lactobacillus Reuteri* ($1x10^9$ cfu/g), *Lactobacillus salivarious* ($1x10^9$ cfu/g), and *Lactobacillus plantarum* ($1x10^9$ cfu/g). Harper et al. (1983) showed that phase 7 pigs fed 1g/kg of *Lactobacillus acidophilus* ($4x10^6$ cfu/g) showed no significantly improvement in daily gain or feed intake. Kornegay and Risley (1996) did not find significant improvements in growing-finishing performance when *Bacillus subtilis* ($1.6x10^9$ cfu/g) and *Bacillus licheniformis* ($1.6x10^9$ cfu/g) were provided as a dietary supplement.

The comparison of research outcomes could be difficult for DFM because its functionality is closely related to the strain specificity, concentration, dosage, administration route, strain viability, and treatment length (Chesson, 1994). According to Lessard et al. (2009) direct-fed microbial efficacy may also be affected by animal age and environment condition.

Direct-fed microbial: Impacts on carcass traits

The commercial sale of finishing pigs is based on grid or matrix of carcass weight and physicochemical meat properties. Premium and discounts are dynamic and may vary according to the carcass attributes (Sellers, 2002). This strategy has increased the weight similarity and quality among carcasses (Gerlemann, 2014) forcing the producers to implement programs that enhance growth performance, feed efficiency, and meat qualitative and quantitative characteristics (Lebret et al., 2014).

Meat preferences changed through the years due to new lean lifestyle. Nowadays, consumers demand high quality and leaner pork, which led the swine production into a new direction. Genetic selection, diet manipulation, and feed additives have improved pork compositional characteristics to met consumer's demand (Cannon et al., 1994). Ractopamine was extensively applied to enhance carcass yield and leanness without altering meat physical-chemical properties (Stoller et al., 2003; Williams et al., 1994; Patience et al., 2009).

In 2006, 160 countries, including the main U.S. pork buyers, have banned or restricted the use of ractopamine. In addition, a considerable number of national retailers stopped buying meat products from animals fed this compound. The ban has increased the interest of DFM. Few researches have tested the influence of the dietary direct-fed microbial supplementation on pork quality (Centner et al., 2014).

Alexopoulos et al. (2003) evaluated the effect of medium and high doses of *Bacillus lincheniformis* ($1.28x10^6$ cfu/g) and *Bacillus subtilis* ($1.92x10^6$ cfu/ g) on carcass quality. The carcass evaluation was based on the subjective European carcass classification model

(SEUROP), which S means superior, E excellent, U very good, R good, O fair and P poor (EEC, 1991). Direct-fed microbial had a significant effect on growing-finishing carcasses, which were classified as superior and excellent. Sudikas et al. (2010) also supplemented growing-finishing pigs with 0.06% of *Bacillus lincheniformis* ($1.6x10^9$ cfu/g) and *Bacillus subtilis* ($1.6x10^9$ cfu/g). In this study, the carcass yield increased by 5.9% and lean meat by 1.72%.

Tian-yang et al. (2013) compared the qualitative properties of *Longissimus dorsi* muscle of growing-finishing pigs fed basal diet and 1% mixture of yeast, lactic acid bacteria and *Bacillus subtilis* (total viable count of $1x10^7$ cfu/g). Direct-fed microbial reduced the drip had no significant effect in muscle pH and color. Černauskienė et al (2011) tested the effect of *Enterococcus faecium* ($10x10^9$ cfu/kg) on carcass quality and meat physicochemical parameters of growing-finishing pigs. Carcass properties and meat quality were not significantly affected by dietary direct-fed microbial supplementation.

Only few studies have been undertaken to analyze the effect of DFM on carcass quality in pigs, however results are not consistent (Alexopoulos et al., 2003; Sudikas et al., 2010; Tian-yang et al., 2013; Černauskienė et al., 2011). Also, due to slight literature about this topic, the correlation of direct-fed microbial and meat quality is still limited.

Direct-fed microbial may balance intestinal microbiota and optimize nutrient utilization causing positive effects on carcass traits. However, its action depends on strain effectiveness, viability, and concentration (Parker, 1974; Fuller, 1989). The variation in protocol models, microbe strains, animal health condition, and farm environment might also influence the outcome variability (Alexopoulos et al., 2003). This present study aimed to

increase awareness of the beneficial effects of different strains on growth performance and carcass traits of growing-finishing pigs

Scope of Thesis

This thesis was based on two studies using DFM as dietary supplements in growing-finishing feeds. The first study included *Lactobacillus acidophilus* strain NP51 ($1x10^9$ cfu/gram) in feeds of growth-finishing pigs (phase 5, 6, and 7 from 45 to 110 kg BW). The second study included a combination of *Lactobacillus acidophilus* ($2.5x10^7$ cfu/g), *Lactobacillus casei* ($2.5x10^7$ cfu/g), *Bifidobacterium thermophilum* ($2.5x10^7$ cfu/g), and *Enterococcus faecium* ($2.5x10^7$ cfu/g) in feeds of finishing pigs (phase 6 and 7 from 89 to 113 kg BW). These studies aimed to investigate the effect of dietary inclusion of mono-strain DFM or multi-strain DFM on growth performance and carcass traits of growing-finishing pigs.

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CHAPTER 2

EFFECT OF DIETARY INCLUSION OF A MONO-STRAIN DIRECT-FED MICROBIAL ON GROWTH PERFORMANCE AND CARCASS TRAITS OF GROWING-FINISHING PIGS

Abstract

This study was to determine the effect of a mono-strain direct-fed microbial (SS-DFM, *Lactobacillus acidophilus* $1x10^9$ cfu/g) on growth performance and carcass traits of growing-finishing pigs. Sixty pigs (30 gilts and 30 barrows) with initial BW of 44.4 kg ± 1.8 kg were allotted to 2 dietary treatments in a randomized complete block design with sex and initial BW as blocks. Experimental diets met the NRC nutrient requirements. The inclusion levels of DFM were 0.28, 0.24, and 0.20% during phase 5 (d 0 to 28), phase 6 (d 28 to 49), and phase 7 (d 49 to 70), respectively. Growth performance was measured for 10 wk. Blood samples were collected on d 63 to measure TNF-α and blood urea nitrogen (BUN). At wk 10, pigs were slaughtered at a local abattoir to evaluate carcass and loin quality including HCW, CCW, backfat thickness (1^{th}, 10^{th}, and last rib), loin weight, loin color (Minolta colorimeter), loin marbling score (1 to 10, NPPC scale), drip loss (48 hr), loin eye area, and nutrient composition. Data were analyzed using Proc Mixed of SAS with treatment and sex as fixed effects and initial BW block as a random effect. The ADG, ADFI, and G:F were not different ($P > 0.10$) between treatments. Backfat thickness of pigs with DFM was smaller ($P < 0.05$, 24.5 vs. 29.6 mm) at the last rib and tended to be smaller ($P = 0.088$, 30.1 vs. 34.4 mm) at the 1^{st} rib than pigs without DFM. Loin of pigs with DFM tended to be redder ($P = 0.084$, 7.22 vs. 6.35 a^* value) than pigs without DFM. Loin nutrient composition, drip loss, TNFα, BUN, and intestinal morphology did not differ between treatments. Collectively, dietary supplementation of mono-strain DFM, *Lactobacillus acidophilus*, to grower-finisher feeds reduced backfat thickness of pigs without affecting growth performance and loin quality.

Key words: Direct-fed microbial, growing-finishing pigs, growth performance, carcass traits, loin quality.

Introduction

World meat production has almost quadrupled from 78 million metric tons in 1963 to 312 million metric tons in 2014 (FAO, 2014). Global pork production has nearly five folded from 1965 to 2014, 24 million metric tons to 116 million metric tons, respectively (FAOSTAT, 2014). Europe and Asian countries are the main pork producers representing 24% and 57%, respectively, of world pork production, whereas North and South America represent only 18% (FAOSTAT, 2014). The United States pork export market expanded discreetly in 2014 due to health concerns, new trade boundaries, and the banned or restriction of antimicrobials and growth-promoter factors applied by the main pork importers, such as the China, Japan, and European Union (FAO, 2014; EFSA, 2015; USDA, 2015; FAO, 2014).

In the past, antimicrobials were widely applied in swine production without veterinary prescription to prevent and treat disease outbreaks, and to enhance growth performance (Maron et al., 2013). However, the new antimicrobial regulation is stimulating producers to find new methods to increase animal production efficiency (Marshall and Levy, 2011; Marchant-Forde et al., 2003).

The dietary supplementation of direct-fed microbial (DFM) could be a valuable alternative to the use of antimicrobial as a growth-promoter factor. Direct-fed microbial is defined as mono- or multi-strain of live microorganism culture that positively influence intestinal microbiota balance and might enhance digestion, immunity, growth performance, and carcass quality of pigs (Fuller, 1992; Meng et al., 2014; Qiu et al., 2012; Suo et al., 2012; Gloria et al., 2010; Chen, et al., 2005; Yang et al., 2014). The genus *Lactobacillus* is the most used DFM for pigs once it is present in large quantities in pigs' gastrointestinal tract. Direct-

fed microbials are usually included during feed batching (Ewing and Cole, 1994; Franklin et al., 2002; Konstantinov et al., 2006).

Lactobacillus acidophilus is one of the main strains included in pig diets (Gomes and Malcata, 1999). Some studies expressed the positive relationship between *Lactobacillus acidophilus* and growth performance of nursery to growing-finishing pigs (Fuller, 1989; Cromwell et al., 1991; Pollman et al., 1980; Choi et al., 2014). However, other experiments have not observed growth enhancement of pigs supplemented with the same *Lactobacillus* strain (Harper et al., 1983; Pollman et al., 1980). The variations might be correlated with the microorganism source, strain viability, DFM preparation, dosage, farm environment condition, and animal age (Chesson, 1994; Lessard et al., 2009; Alexopoulos et al., 2003).

This study was performed to test the hypothesis that mono-strain DFM possibly enhance nutrient uptake and, consequently, increase muscle deposition, growth performance, and carcass traits of growing-finishing pigs, which are crucial factor for animal production profitability. Therefore, the objective of this study was to determine the effects of the dietary supplementation of a mono-strain DFM based on *Lactobacillus acidophilus* on growth performance and carcass traits of growing-finishing pigs.

Materials and Methods

The animal experimental protocol used in this study was approved by North Carolina University Animal Care and Use Committee.

Animals and Housing

A total of 60 growing-finishing pigs (Smithfield Premium Genetics, Rose Hill, NC) with initial BW of 44.4 ± 1.8 kg were housed in pens (3.65x1.37 meters) for a 10-wk experiment at the North Carolina Swine Evaluation Station (Clayton, NC). The dietary treatments were control (CON, basal diet with no *Lactobacillus acidophilus*) and mono-strain DFM (DFM, basal diet supplemented with *Lactobacillus acidophilus*) (Table 1). Treatments were allotted in a randomized complete block design with initial body weight and sex as blocks. Each treatment had 10 pens (5 gilt pens and 5 barrow pens) with 3 pigs per pen. Pens had solid concrete floor and were equipped with one self-feeder and a nipple water to allow ad libitum access to feed and water throughout the experimental period. Pigs were fed experimental diets for 10 wk, which were divided on 4 wk for phase 5 (d 0 to 28), 3 wk phase 6 (d 28 to 49) and 3 wk phase 7 (d 49 to 70). Body weight and feed intake were measured weekly on d 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70 for growth performance evaluation (ADG, ADFI, and G:F) (Table 2).

Experimental Diets

During this study, DFM and CON diets had corn, soybean meal and DDGS as major ingredients. In addition, DFM diets were supplemented with mono-strain of direct-fed microbial (*Lactobacillus acidophilus* 1 x 10^9 cfu/g, NPC, Guymon, OK). The inclusion levels of DFM were 0.28, 0.24, or 0.20% for phase 5, 6, and 7, respectively. Experimental diets

were batched at North Carolina State University Feed mill (Raleigh, NC) and met the concentration and nutrient requirements suggested by NRC (2012). The diet nutrient composition was analyzed by North Carolina Department of Agriculture (Raleigh, NC) (Table 1).

Blood Sampling and Measurements

On d 66, a total of 20 pigs representing the medium body weight of each pen were bled from the jugular vein. A 10 mL vacutainer with no anticoagulant (BD, Franklin Lakes, NJ) was used to obtain serum samples for cytokine and urea nitrogen measurements. Blood samples were centrifuged at 3,000 x g for 15 min at 4°C and 2 mL of serum were stored at -80°C.

The serum samples were used to measure possible occurrence of systemic inflammation (TNFα) by the porcine TNFα colorimetric ELISA kit (Pierce Biotechnology, Inc., Rockford, IL). The procedure consisted of the addition of 50 µL of standard solution and 100 µL of diluted serum in microplate wells coated with capture antibody and biotinylated antibody reagent followed by consecutives washes and detection. The detection procedure involved the introduction of horseradish peroxidase, TMB substrate and a stop solution. The microplate absorbance was read at 450 nm and 540 nm using an ELISA plate reader combined with KC4 data analysis software. The TNFα detection limit was 5 pg/mL.

Blood urea and nitrogen (BUN) was measured using the liquid urea nitrogen reagent set (Pointe Scientific, Inc., Canton, MI) to evaluate nitrogen excretion. The procedure was basically the combination of 1 mL of working reagent based on urease and α-Ketoglutarate with 10 µL of standard solution (Urea nitrogen 30 mg/dL) or 10 µL of serum. After mixed,

samples were immediately placed into a spectrophotometer reader (Genesys 10 UV) where A1 and A2 absorbance values were recorded at 30 secs and 60 secs, respectively. Blood urea nitrogen was determined by the followed formula: [(A1 - A2 serum)/(A1 - A2 standard)] x Standard Concentration. Expected values of BUN were 7 to 18 mg/dL.

Handling and Slaughtering

At the end of the experiment, a pig from each pen representing the medium body weight was selected and slaughtered at a local commercial abattoir (Bailey Foods, Spring Hope, NC). Pigs were slaughtered using percussive stunning and exsanguination, and identified with their respective identification numbers. Slaughter technique complied with national regulations (FSIS, 2013).

Carcass Traits

After the slaughter, hot carcass weight (HCW) was recorded. The dressing percentage was calculated by the ratio of HCW and final live BW. Carcasses were chilled for 24 hr at 4°C, then, cold carcass weight (CCW) was acquired to calculate cooling loss through the ratio of CCW and HCW. Using a ruler, dorsal subcutaneous adipose tissue thickness was measured in millimeters at the first, tenth, and last ribs. Boneless loin was removed from the right side of each carcass, immediately identified, and weighed. A 250 g loin section was removed from each loin for color and marbling evaluation, loin eye area (LEA), loin chemical composition analysis, and drip loss determination. Carcass traits are presented in Table 3, 4, and 5.

Meat color was evaluated using a color panel scale of 1 to 6 according to National Pork Producers Council (1991) standards and objectively by the collection of lightness (l*),

redness (a*), and yellowness (b*) values of cranial, medial, and caudal portion of each loin section (Model CR-410 Chromameter, Konica Minolta Sensing Inc., Osaka, Japan). The marbling was subjective scored using a marbling scale of 1 to 10 according to National Pork Producers Council (1991) guideline. The surface of each loin eye section was drawn on a transparency paper and loin eye area (LEA) was measured with a grid paper in square centimeters. A 50 g portion of the each loin section was designated to chemical composition analysis to determine meat dry matter, ether extract, ash, and crude protein following AOAC (1990) procedures. A slice of about 3-cm of each loin eye section was weighed (W1), hanged into a sealed polyethylene plastic bag and placed into a 4°C refrigerator. After 48 hr, each slice was gently dried, weighed (W2) and the drip loss was calculated by the difference between W1 and W2 (Dribi et al., 2014).

Intestinal Sampling

Just after the slaughter, the gastrointestinal tract was quickly removed and identified. Proximal portion of jejunum and colon of each pig were collected and 7-cm sections were obtained. Each section was gently flushed, submerged into a 10% formaldehyde-phosphate buffer, and individually identified for further microscopic morphology evaluation. The distal portion of jejunum and colon were also obtained to collect 1.5 mL of mucosal samples, which were immediately placed in liquid nitrogen for short period and transferred to -80°C freezer until TNFα measurement (Zhao et al., 2014).

Intestinal Morphology

The formalin-fixed jejunum and colon sections were trimmed and placed in cassettes. The North Carolina State University Histopathology Laboratory (College of Veterinary

Medicine, Raleigh, NC) embedded the samples in paraffin, thin-sectioned, and secured them on microscope slides with hematoxylin and eosin staining. The slides were evaluated under a Sony CCD video camera attached to a microscope with 40x magnification. An average of 10 villus height, villus width, and crypt depth were measured in each slide following the methodology described by Shen et al. (2009).

Mucosal Cytokine Measurement

The tumor necrosis factor α (TNFα) was also measured in jejunum and colon mucosal samples. A range of 0.50 to 0.51 g of mucosa was homogenized with 1 mL of PBS solution, ground, and centrifuged for 10 min at 15,000 g. A 2 mL of supernatant was obtained for total protein calculation by BCA assay (Peace et al., 2011). After total protein measurement, TNFα was obtained by porcine TNFα colorimetric ELISA kit (Pierce Biotechnology, Inc., Rockford, IL). Concentrations of TNFα were expressed in pg/mg of total protein (Table 6).

Statistical Analysis

The experiment used a randomized complete block design having sex and treatment as fixed effects and initial body weight blocks as a random effect. For growth performance (BW, ADG, ADFI, and G:F) analysis, pen was considered the experimental unit. The remaining measurements had the harvested pigs (one per pen) as the experimental unit. All data were analyzed by Proc Mixed of SAS 9.3 (SAS Institute Inc., Cary, NC). A probability level of $P < 0.05$ was considered statistically significant whereas the probability of $0.05 \leq P < 0.10$ was considered trend.

Results

Growth Performance

Initial BW did not differ ($P > 0.10$) among treatments (Table 2). The dietary supplementation of DFM did not affect ($P > 0.10$) ADG during phase 5 and overall. The dietary supplementation of DFM decreased ($P < 0.05$) ADG (by 11%, 1.03 to. 0.92 kg/d) during the first week of phase 6 and tended to decrease ($P = 0.080$) ADG (by 7%, 1.1 vs. 1.0 kg/d) during the second week of phase 7.

The dietary supplementation of DFM did not affect ($P > 0.10$) ADFI during phase 5, 6, and overall. However, the supplementation of DFM tended to decrease ($P = 0.072$) ADFI (by 6%, 3.869 to 3.644 kg/d) during the first week of phase 7.

The dietary supplementation of DFM did not affect ($P > 0.10$) G:F during phase 5, phase 7, and overall. However, the supplementation of DFM tended to increase ($P = 0.091$) G:F (by 24%, 0.272 to 0.337) during the second week of phase 6.

Carcass Traits

Results for the carcass traits of DFM and CON treatments are presented on Table 3. The dietary supplementation of DFM did not affect ($P > 0.10$) hot carcass weight, cold carcass weight, cooling loss, dressing percentage, loin weight, loin eye area, and the 10^{th} rib backfat thickness. The dietary supplementation of DFM tended to decrease ($P = 0.088$, 30.1 vs. 34.4 mm) backfact thickness from the first rib whereas the dietary supplementation of DFM decreased ($P < 0.05$, 24.5 vs. 29.6 mm) backfat thickness from the last rib.

Loin Composition

The loin nutrient composition is present on Table 4. The dietary supplementation of DFM did not affect ($P > 0.10$) the percentage of DM, ether extract, ash, and CP of growing-finishing pigs.

Meat Quality

The meat quality is presented in Table 5. The dietary supplementation of DFM did not affect ($P > 0.10$) the drip loss (DL %), lightness (l*), yellowness (b*), and marbling (LM) of growing-finishing pigs. However, loin from DFM treatment tended to be redder in color ($P = 0.084$, 7.22 vs. 6.34 a*).

Inflammatory Cytokine and Blood Urea Nitrogen

The tumor necrosis factor alpha and blood urea nitrogen values are present on Table 6. The dietary supplementation of DFM did not affect ($P > 0.10$) TNFα and blood urea nitrogen of growing-finishing pigs.

Intestinal Morphology Evaluation

The intestinal morphology measurements are present on Table 7. The dietary supplementation of DFM did not affect ($P > 0.10$) villus height, villus width, and crypt of growing-finishing pigs.

Discussion

Growth Performance

The current study showed that the dietary supplementation of mono-strain DFM did not enhance average daily gain, average daily feed intake, and feed efficiency of growing-finishing pigs. Similar results were observed by Pollman et al. (1980), Harper et al. (1983), and Kornegay et al. (1990), which the supplementation of *Lactobacillus acidophilus* DFM did not affect the growth performance of growing-finishing pigs. According Kiesling et al. (1982), Salarmoini and Fooladi (2011), Vasconcelos et al. (2008), and Peterson et al. (2007) growth performance of finishing steers and broiler were also not enhanced by *Lactobacillus acidophilus* dietary supplementation.

However, these results are contrary to some studies that use different species and strains of *Lactobacillus* sp. as DFM. Suo et al. (2012) demonstrated that the supplementation of *Lactobacillus plantarum* improved ADG and G:F of growing pigs. Shon et al. (2005) found that a mixture of *Lactobacillus reuteri*, *Lactobacillus salivarus*, and *Lactobacillus plantarum* included in low and high energy diets enhanced ADG, ADFI, and G:F of growing-finishing pigs. Also, Elam et al. (2003) and Jin et al. (1998) observed that different cultures of *Lactobacillus* enhanced the growth performance of finishing beef steers and broilers, respectively.

Although mono-strain DFM beneficial effects on growth performance are not always observed, according to this current study and the literature, it is clear that *Lactobacillus* does not produce any negative effects on growth performance. The inclusion of DFM on pig's diets might improve growth performance due to competitive pathogenic inhibition in the gut,

the release of antibacterial substances, immunity promoter effects, and the increase of nutrient uptake (Krehbiel et al., 2003). However, the DFM efficacy depends directly on several other factors, such as strain viability, dose, application method, frequency, DFM storage, environmental condition, and host health status and age (Chesson, 1994; Lessard, et al., 2009).

There are three possible explanations to clarify the lack of DFM response on the growth performance in this study. First, growing-finishing pigs may have developed a stable and mature gut microbiota, which might be difficult to alter in order to increase nutrient uptake even with long periods of DFM dietary supplementation (Konstantinov et al, 2006; Suo et al., 2012). Second, knowing DFM effects are genera, specie, and strain dependent, it may suggest that mono-strain DFM is less functional than multi-strain DFM (Sanders and Huis In't Veld, 1999). Different from mono-strain DFM, multi-strain DFM may have its functionality enhanced due to synergist and symbiotic effects produced on the host through the sum of each strain benefit (Campieri and Giochetti, 1999; Timmerman et al., 2004). In addition, studies showed that the gut colonization and activity of *Lactobacillus acidophilus* could be enhanced by the presence of *Bifidobacterium spp*. (Gomes et al, 1998; Kailasapathy and Chin, 2000). Third, DFM supplementation seems to generate more benefits on livestock under stress conditions once host immunity can be enhanced by DFM (Lyons, 1987; Jin et al., 1997). In this study, pigs were kept under essentially stress-free condition with optimal temperature ranges, ad-libitum access to water and feed, and no disease outbreak.

Carcass Traits

In the past, the major goal of the swine industry was to increase carcass meat percentage of growing-finishing pigs (Cameron, 1990). Nowadays, due to changes in marketing demands and consumer preferences, animal producers started to focus on different strategies to produce high quality carcass traits and, at the same time, keep low production cost with restricted or no inclusion of antimicrobial and growth-promoter factors (Newcom et al., 2004). Backfat thicknesses and loin color are one of the variables used to predict carcass trait quality (Schinckel et al., 2009). Backfat thicknesses may be correlated to loin marbling and carcass leanness (Sonesson et al., 1998; Moddy and Zobrisky, 1966) whereas color is directly associated with buyers' first sensorial impression and meat quality (Meng et al., 2014). Many questions have been raised regarding the positive relationship between DFM and carcass traits, but its application still questioned in result of inconstant outcomes.

In this current study, slight differentiation of carcass traits between treatments was observed. The inclusion of mono-strain DFM tended to reduce the backfat thickness of the first rib and reduced the backfat thickness of the last rib. With the limited literature correlating *Lactobacillus acidophilus* and backfat thicknesses of growing-finishing pigs, this study only agreed with one other experiment. According to Kim et al. (1998), finishing pigs fed a diet based on sorghum-soybean meal and *Lactobacillus acidophilus* showed smaller backfat thickness on the last rib.

This result may be explained by the association of *Lactobacillus* with lipolytic effects. One of the direct-fed microbial attributes is the beneficial effect on the nutritional efficiency of the host through the hormone secretion modification (Guarner et al., 2003; Cani

et al, 2009). Some studies reported that different species of *Lactobacillus* inhibit lipoprotein lipase (Matsunara et al., 2010; Yonejima et al., 2013; Yokota et al., 2010). According to Yasunori (2013), *Lactobacillus* may react directly with LPL preventing it to bind substrates. The lipoprotein lipase regulates tryglicerol storage and fat levels deposited in the adipose tissue of growing-finishing pigs (Kris-Etherton and Etherton, 1982; Loh et al., 2001). Therefore, assuming *Lactobacillus* inhibits LPL activity, it might influence the fat deposition rate and possibly reduce backfat deposition. However, further investigation regarding LPL levels in pigs fed DFM is required.

Inflammatory Cytokine and Blood Urea Nitrogen

Cytokines are pro-inflammatory mediators, which regulate immune and inflammatory events. The gut epithelial cells constitute a physical barrier that prevents pathogen invasion and produce a cascade of signs based on cytokines that are decisive to prevent infections and to maintain host homeostasis (Pie et al., 2004). TNF-α is one of the cytokines released by normal epithelial cells but significantly regulated in microbial infection by the activation of macrophages, monocytes, and some lymphocytes (Jung et al., 1995; Pauli, 1995). Nutrition profile might alter cytokines levels suggesting that the inclusion of DFM may affect the gut TNF-α production (Isolauri et al., 2001). Besides the influence on inflammatory and immune responses, TNF-α showed to influence the turnover rate of gut cells due to its high mitogenic potential in the small intestine (Galy et al., 1989; Muller et al., 1996; Zachrisson et al., 2001).

In the current study, TNF-α concentration in serum and mucosal samples did not differ between treatments. It suggested that DFM and CON pigs did not respond to acute or chronic infections being considered healthy during the 10 wk experiment. According to the

current intestinal morphology results, the TNF-α levels present in this trial did not affect small intestinal epithelium cells.

Another factor that could be influenced by DFM supplementation is the gut nitrogen digestibility or uptake due to DFM beneficial effects in the gut microbiota. According to Bradford (2014) the blood and urea nitrogen is a usual approach to measure the levels of amino acid oxidation and nitrogen excretion. Urea is derived from ammonia, which is a product of amino acid catabolism and it is mainly produced in the liver. When urea reaches the intestine, it is broken down by urease, which is released by gut microbiota, and then, the nitrogen is recycled to the liver or excreted. Nursing animals tended to have lower levels of BUN due to anabolic stage. However, high levels of BUN may suggest acute and chronic infections, which increase amino acid oxidation.

In this study, BUN did not differ between the treatments indicating that the inclusion of DFM did not affect nitrogen digestibility or increase amino acid oxidation due to good health status of pigs.

Intestinal Morphology

The intestinal morphology reveals gut health status (Choi et al., 2014). Villus height and crypt depth may play an important rule against pathogenic bacteria invasion and toxic absorption in the intestinal lumen (Paul et al., 2007). Several studies reported that DFM increased villus height in piglets (Mourao et al., 2005; Willing and Van Kessel, 2007; Di Giancamillo et al., 2008; Yang et al., 2009). Other studies showed that the supplementation of *Lactobacillus* enhanced villus height and crypt depth of weanling and growing-finishing pigs (Choi et al, 2014; Suo et al., 2012; Willing and Van Kessel, 2007). Yang et al. (2009)

reported that crypt depth decreased in mice supplemented with moderate or high doses of DFM and increase with the supplementation of lower dose of the same DFM.

In current study, the dietary supplementation of mono-strain DFM did not affect villus height and crypt depth of growing-finishing pigs, which contraries most of the literature. It may be explained by the fact that the effect of DFM on villus height and crypt depth depends on the DFM specie and strain (Matur and Eraslan, 2012). Also, the age of pigs may influence the efficiency of DFM on intestinal modulation once growing-finisher pigs showed to have a more stable and mature gut microbiota (Konstantinov et al., 2006; Suo et al., 2012).

Conclusion

Collectively, the supplementation of 0.28, 0.24, and 0.20% of *Lactobacillus acidophilus* ($1x10^9$ cfu/g) did not affect the growth performance, serum and mucosa cytokine levels, blood and urea nitrogen, and meat quality of growing-finishing pigs, but decreased the backfat thickness from the first and last rib indicating that the supplementation of *Lactobacillus acidophilus* may inhibit lipoprotein lipase. However, further research is required to answer this new hypothesis.

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Table 1. Composition of DFM and CON diets (Phase 5: d 0 to 28, Phase 6: d 28 to 49, and Phase 7: d 49 to 70).

	Phase 5		Phase 6		Phase 7	
Item	**CON**	**DFM**	**CON**	**DFM**	**CON**	**DFM**
Ingredient, %						
Yellow corn	69.43	69.43	74.15	74.15	77.92	77.92
Soybean meal	17.28	17	12.74	12.50	9.2	9
DDGS	10	10	10	10	10	10
L-Lys HCl	0.27	0.27	0.26	0.26	0.22	0.22
L-Thr	0.03	0.03	0.03	0.03	0.02	0.02
Salt	0.22	0.22	0.22	0.22	0.22	0.22
Vitamin premix[2]	0.03	0.03	0.03	0.03	0.03	0.03
Mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15
Dical P (26Ca18P)	0.6	0.6	0.5	0.5	0.4	0.4
Limestone	0.99	0.99	0.92	0.92	0.84	0.84
Poultry fat	1	1	1	1	1	1
DFM[1]	0	0.28	0	0.24	0.00	0.20
Calculated composition						
DM, %	89.08	89.08	88.99	88.99	88.9	88.9
ME, Mcal/kg	3.360	3.360	3.370	3.370	3.379	3.379
CP, %	16.98	16.98	15.19	15.19	13.77	13.77
Lys, %	0.85	0.85	0.73	0.73	0.61	0.61
Cys + Met, %	0.50	0.48	0.46	0.46	0.43	0.43
Trp, %	0.15	0.15	0.13	0.13	0.11	0.11
Thr, %	0.52	0.52	0.46	0.46	0.4	0.4
Ca, %	0.59	0.59	0.52	0.52	0.46	0.46
Available P, %	0.27	0.27	0.24	0.24	0.21	0.21
Total P, %	0.47	0.47	0.22	0.22	0.4	0.4
Analyzed composition						
DM, %	91.89	92.01	91.53	91.21	91.93	91.17
CP, %	15.02	16.17	15.17	14.86	14.31	15.09
ADF, %	3.78	3.85	3.48	2.93	3.59	3.35
Ca, %	0.56	0.53	0.65	0.50	0.55	0.59
P, %	0.45	0.50	0.45	0.43	0.45	0.43

Table 1. Continued

[1]:*Lactobacillus acidophilus* 1 x 10^9 cfu/gram.

[2]:Vitamin premix provided per kilogram of complete diet: 22,045,000 IU of vitamin A; 3,306,900 IU of vitamin D_3; 66,138 IU of vitamin K; 88 mg of vitamin B_{12}; 15,432 mg of riboflavin; 88,184 mg of niacin; 61,729 mg of d-pantothenic acid; and 220 mg of biotin.

[3]: Mineral premix provided the following composition: 1.10% of Cu; 198.0 mg/kg of I; 11.02% of Fe; 2.64% of Mn; 198.4 mg/kg of Se; and 11.02% of Zn.

Table 2. Growth performance of growing-finishing pigs fed diets supplemented with DFM.

	Treatment			
Item	**CON**	**DFM[1]**	**SEM**	***P* value**
BW, kg				
Initial	44.4	44.4	1.8	0.974
Wk 1 (Phase 5)	51.6	51.5	2.0	0.962
Wk 2 (Phase 5)	59.3	59.4	2.2	0.927
Wk 3 (Phase 5)	68.8	68.6	2.3	0.761
Wk 4 (Phase 5)	74.6	74.3	2.3	0.668
Wk 5 (Phase 6)	81.8	80.8	2.4	0.094
Wk 6 (Phase 6)	86.9	86.8	2.7	0.942
Wk 7 (Phase 6)	92.4	91.8	2.7	0.436
Wk 8 (Phase 7)	98.0	96.9	2.8	0.206
Wk 9 (Phase 7)	106.0	104.3	2.9	0.133
Wk 10 (Phase 7)	113.4	111.2	3.3	0.145
ADG, kg/d				
Wk 1 (Phase 5)	1.021	1.018	0.043	0.963
Wk 2 (Phase 5)	1.110	1.122	0.053	0.852
Wk 3 (Phase 5)	1.355	1.316	0.029	0.270
Wk 4 (Phase 5)	0.826	0.814	0.031	0.794
Wk 5 (Phase 6)	1.035	0.923	0.046	0.036
Wk 6 (Phase 6)	0.722	0.866	0.078	0.218
Wk 7 (Phase 6)	0.790	0.714	0.072	0.363
Wk 8 (Phase 7)	0.788	0.719	0.088	0.511
Wk 9 (Phase 7)	1.142	1.067	0.047	0.080
Wk 10 (Phase 7)	1.058	0.986	0.082	0.418
Overall	0.985	0.955	0.029	0.159
ADFI, kg/d				
Wk 1 (Phase 5)	2.211	2.166	0.104	0.466
Wk 2 (Phase 5)	2.668	2.505	0.121	0.288
Wk 3 (Phase 5)	3.041	2.97	0.173	0.735
Wk 4 (Phase 5)	2.456	2.592	0.120	0.441
Wk 5 (Phase 6)	3.509	3.415	0.100	0.327
Wk 6 (Phase 6)	2.623	2.576	0.132	0.717
Wk 7 (Phase 6)	2.406	2.306	0.123	0.283
Wk 8 (Phase 7)	3.869	3.644	0.098	0.072
Wk 9 (Phase 7)	3.155	3.028	0.125	0.286
Wk 10 (Phase 7)	2.325	2.246	0.158	0.552
Overall	2.826	2.745	0.084	0.123

Table 2. Continued

G:F				
Wk 1 (Phase 5)	0.465	0.473	0.015	0.721
Wk 2 (Phase 5)	0.424	0.451	0.022	0.355
Wk 3 (Phase 5)	0.464	0.45	0.027	0.721
Wk 4 (Phase 5)	0.347	0.318	0.022	0.377
Wk 5 (Phase 6)	0.294	0.269	0.008	0.047
Wk 6 (Phase 6)	0.272	0.337	0.025	0.091
Wk 7 (Phase 6)	0.33	0.308	0.029	0.512
Wk 8 (Phase 7)	0.205	0.196	0.023	0.764
Wk 9 (Phase 7)	0.363	0.354	0.011	0.183
Wk 10 (Phase 7)	0.455	0.437	0.018	0.504
Overall	0.362	0.359	0.004	0.606

[1]:Lactobacillus acidophilus strain $1x10^9$ cfu/gram.

Table 3. Hot carcass weight (HCW), cold carcass weight (CCW), cooling loss (CL), dressing (DRESS), loin eye area (LEA), loin weight (LW), backfat thickness at 1st rib (BTx1), backfat thickness at 10th rib (BTx10), and backfat thickness at last rib (BT x LR) of growing-finishing pigs fed DFM.

	Treatment			
Item	**CON**	**DFM[1]**	**SEM**	***P* value**
Final BW (kg)	119.60	114.40	4.28	0.093
HCW (kg)	90.49	92.44	2.60	0.354
CCW (kg)	86.22	87.49	2.70	0.530
CL (%)	4.72	5.38	0.89	0.615
DRESS (%)	77.42	78.95	0.77	0.133
LW (kg)	7.23	7.12	0.28	0.588
LEA (cm^2)	56.20	57.20	3.66	0.835
BTx1 (mm)	34.40	30.10	2.07	0.088
BTx10 (mm)	23.20	22.20	1.60	0.529
BTxLR (mm)	29.60	24.50	1.84	0.003

[1]: *Lactobacillus acidophilus* $1x10^9$ cfu/gram.

Table 4. Chemical composition of *Longissimus dorsi* muscle of growing-finishing pigs fed DFM.

	Treatment			
Item	**CON**	**DFM[1]**	**SEM**	**P value**
DM	27.83	27.03	0.57	0.263
Fat DM Basis%	12.95	12.71	2.01	0.905
Fat As-is%	3.68	3.51	0.62	0.792
Ash DM Basis%	3.97	4.00	0.12	0.834
Ash as-is%	1.10	1.08	0.02	0.556
CP DM Basis%	75.49	78.86	1.80	0.217
CP As-is%	20.92	21.31	0.60	0.661

[1]:*Lactobacillus acidophilus* of $1x10^9$ cfu/gram.

Table 5. Drip loss (DL), lightness (L*), redness (A*), yellowness (B*), and loin marbling (LM) of *Longissimus dorsi* muscle of growing-finishing pigs fed DFM.

	Treatment			
Item	**CON**	**DFM[1]**	**SEM**	***P* value**
DL (%)	7.43	6.60	0.52	0.297
L*	53.05	52.93	0.88	0.967
A*	6.34	7.22	0.32	0.084
B*	4.77	5.25	0.32	0.269
LM	2.10	2.60	0.29	0.256

[1]:*Lactobacillus acidophilus* $1x10^9$ cfu/gram.

Table 6. TNFα blood, TNFα jejunum, TNFα colon and BUN of growing-finishing pigs fed DFM.

	Treatment			
Item	**CON**	**DFM[1]**	**SEM**	***P value***
TNFα Blood (pg/mL)	73.77	87.27	11.00	0.408
TNFα Jejunum (pg/mg of protein)	0.39	0.28	0.10	0.481
TNFα Colon (pg/mg of protein)	1.55	1.53	0.22	0.959
BUN (mg/dL)	8.91	9.02	1.11	0.945

[1]: *Lactobacillus acidophilus* of $1x10^9$ cfu/gram.

Table 7. Villus height, width, crypt depth and villus height to crypt depth ratio from jejunum and colon of growing-finishing pigs fed DFM.

	Treatment			
Item	**CON**	**DFM[1]**	**SEM**	***P* value**
Jejunum (µm)				
Villus height	302.83	302.94	14.25	0.996
Villus width	142.87	134.74	5.64	0.335
Crypt depth	196.17	191.77	8.50	0.723
VCR[3]	1.54	1.61	0.09	0.620
Colon (µm)				
Crypt depth	322.08	346.14	14.69	0.257

[1]: Lactobacillus acidophilus $1x10^9$ cfu/gram.
[3]: Villus height to crypt depth ratio.

CHAPTER 3

EFFECT OF DIETARY INCLUSION OF MULTI-STRAIN DIRECT-FED MICROBIAL ON GROWTH PERFORMANCE AND CARCASS TRAITS OF GROWING-FINISHING PIGS

Abstract

This study was to determine the supplemental effect of multi-strain direct-fed microbial (DFM, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum,* and *Enterococcus faecium* $1x10^8$ cfu/g) on growth performance and carcass traits of growing-finishing pigs. Sixty pigs (30 gilts and 30 barrows) with initial BW of 88.5 ± 0.6 kg were allotted to 2 dietary treatments in a randomized complete block design with sex and initial BW as blocks. Experimental diets met NRC nutrient requirements. The DFM inclusion level was 0.05% during phase 6 (d 0 to 14) and phase 7 (d 14 to 28). Growth performance was measured for 4 wk. Blood samples were collected on d 21 to measure TNFα and blood urea nitrogen (BUN). At wk 4, pigs were harvested at a local abattoir to evaluate carcass and loin quality including HCW, CCW, backfat thickness (1^{th}, 10^{th}, and last rib), loin weight, loin color (Minolta colorimeter), loin marbling score (1 to 10, NPPC scale), drip loss (48 hr), loin eye area, and loin chemical composition. Data were analyzed using Proc Mixed of SAS with treatment and sex as fixed effects and initial BW block as a random effect. The dietary supplementation of DFM increased ($P < 0.05$) ADFI (by 9%, 2.56 to 2.79 kg/d) and tended to increase ($P = 0.062$) ADG (by 18%, 0.753 to 0.889 kg/d). The inclusion of DFM also increased ($P < 0.05$) hot carcass weight (92.75 vs. 85.32 kg), cold carcass weight (88.9 and 81.7 kg), and dressing % (78.8% and 77.3%) of growing-finishing pigs. Loin nutrient composition, loin color, loin marbling, loin weight, backfat thicknesses, drip loss, TNFα, BUN, and intestinal morphology did not differ between treatments. Collectively, dietary supplementation of multi-strain DFM, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum,* and *Enterococcus faecium*, to grower-finisher feeds

enhanced feed intake, and consequently, increased carcass yield and tended to increase growth without affecting loin quality.

Key words: Direct-Fed microbial, growing-finishing, growth performance, carcass traits, loin quality

Introduction

Antimicrobials have been extensively applied in swine production to treat and prevent disease outbreaks and enhance growth-performance in the United States with no veterinary prescription since 1951 (Jones and Ricke, 2003; Hahn et al., 2006). In addition, since 2001, major swine producers have supplemented pigs with beta-adrenergic growth-promoter factors to obtain leaner cuts, increase pigs' weight gain and protein synthesis, and decrease production length (ARS, 2004).

However, the United States Food and Drug Administration established a new antimicrobial regulation to phase out the use of important medical antimicrobials in food production purposes (FAO, 2013). In addition, some U.S. pork importers have banned or restricted the use of ractopamine in pig production (EFSA, 2009; USDA, 2015). Therefore, to maintain high levels of production with restricted or no use of antimicrobials and growth-promoter factors represent a challenge for producers, who are interested in viable alternatives. The multi-strain direct-fed microbial (DFM) might be an alternative for animal production industry. Defined by Fuller (1989) as a live microbial feed supplement which beneficially affects the host by improving intestinal microbiota, DFM has taken the attention of many scientific studies.

Multi-strain direct-fed microbial showed to be more effective than mono-strain DFM due to widely effects based on genera, species, and strain (Sanders and Huis in't Veld, 1999). The genus *Lactobacillus* is extensively included in DFM formulas because its non-pathogenic characteristics and also for being the main bacteria in pigs' gut microbiota (Ewing and Cole, 1994; Franklin et al., 2002; Konstantinov et al., 2006). The

Bifidobacterium strains are often included in conventional yogurt recipes due to beneficial effects against infantile diarrhea, lactose intolerance, colorectal cancer, and constipation (Orrhage et al., 1994; Jiang et al., 1996; Pool-Zobel et al., 1996; Kleesen et al., 1997). The use of this strain has showed similar developments in animal industry. Pigs fed *Bifidobacterium thermophilum* combated *Salmonella* and *E.coli* infection more efficiently than pigs fed basal diet (Tanner et al., 2014; Sun et al., 2014). The *Enterococcus* genus has also been applied in pig production due to ability to survive at low pH environment, increase immunity, and prevent piglets' from major infections (Macha et al., 2004; Pollmann et al., 2005; Taras et al., 2006;).

Although mode of action is still unknown and benefits as the ones cited above are not always consistent, studies suggested that the inclusion of multi-strain DFM in basal diets might increase growth performance, feed intake, and feed efficiency of growing-finishing pigs (Choi et al., 2014; Giang et al., 2011). The supplementation of multi-strain DFM seems to enhance carcass and pork quality, and consequently, increase carcass yield, meat leanness, and reduce drip loss (Alexopoulos et al., 2003; Tian-yang et al., 2013).

This study was performed to test the hypothesis that multi-strain DFM possibly enhance nutrient uptake and, consequently, increase muscle deposition, growth performance, and carcass traits of growing-finishing pigs, which are crucial factors for animal producer's profitability. Therefore, the objective of this study was to determine the effects of the dietary supplementation of multi-strain direct-fed microbial based on *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* on growth performance and carcass traits of growing-finishing pigs.

Materials and Methods

The animal experimental protocol used in this study was approved by North Carolina University Animal Care and Use Committee.

Animals and Housing

The experiment was conducted at the North Carolina Swine Evaluation Station (Clayton, NC). A total of 60 growing-finishing pigs (Smithfield Premium Genetics, Rose Hill, NC) with initial BW of 85.5 ± 0.6 kg were housed in pens (3.65x1.37 meters) for 4-wk. The dietary treatments were control (CON, basal diet with no multi-strain DFM supplementation) and multi-strain DFM (DFM, basal diet supplemented with *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium*). Treatments were allotted in a randomized complete block design with initial body weight and sex as blocks. Each treatment had 10 pens (5 gilt pens and 5 barrow pens) with 3 pigs per pen. Pigs were fed experimental diets for 4 wk, which were divided on 2 wk for phase 6 (d 0 to 14) and 2 wk phase 7 (d 14 to 28). Pigs had ad libitum access to feed and water throughout the experimental period. Body weight and feed intake were measured weekly on d 0, 14, 21, and 28 for growth performance evaluation (ADG, ADFI, and G:F) (Table 2).

Experimental Diets

Direct-fed microbial and CON diets were batched at North Carolina State University Feed mill (Raleigh, NC). Both experimental diets had corn, soybean meal, and DDGS as major ingredients and met the concentration and nutrient requirements suggested by NRC (2012). Direct-fed microbial diets were supplemented with a multi-strain DFM (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and

Enterococcus faecium, $1x10^8$ cfu/g, Star Labs/ Forage Research Inc., Clarksdale, MO). The inclusion level of DFM was 0.05% during phase 6 and 7. The experimental diets were analyzed by North Carolina Department of Agriculture (Raleigh, NC) (Table 1).

Blood Sampling and Measurements

On d 21, blood samples from 20 pigs representing the medium body weight of each pen were collected. Blood was obtained using a 10 mL vacutainer with no anticoagulant (BD, Franklin Lakes, NJ). All samples were centrifuged at 3,000x g for 15 min at 4°C and 2 mL of serum were stored at -80°C.

The serum samples were used to measure possible occurrence of systemic inflammation (TNFα) by the porcine TNFα colorimetric ELISA kit (Pierce Biotechnology, Inc., Rockford, IL). The procedure consisted in the addition of 50 µL of standard solution and 100 µL of diluted serum in microplate wells coated with capture antibody and biotinylated antibody reagent followed by consecutives washes and detection. The detection procedure involved the introduction of horseradish peroxidase, TMB substrate and a stop solution. The microplate absorbance was read at 450 nm and 540 nm using an ELISA plate reader combined with KC4 data analysis software. The TNFα detection limit was 5 pg/mL.

Blood urea nitrogen (BUN) was measured using the liquid urea nitrogen reagent set (Pointe Scientific, Inc., Canton, MI) to evaluate nitrogen excretion. The procedure was basically the combination of 1 mL of working reagent based on urease and α-Ketoglutarate with 10 µL of standard solution (Urea nitrogen 30 mg/dL) or 10 µL of serum. After mixed, samples were immediately placed into a spectrophotometer reader (Genesys 10 UV) where A1 and A2 absorbance values were recorded at 30 secs and 60 secs, respectively. BUN was

determined by the followed formula: [(A1 - A2 serum)/(A1 - A2 standard)] x Standard Concentration. BUN expected values were 7 to 18 mg/dL.

Handling and Slaughtering

At the end of the experiment, a pig from each pen representing the medium body weight was selected and slaughtered at a local commercial abattoir (Bailey Foods, Spring Hope, NC). Pigs were slaughtered using percussive stunning and exsanguination, and identified with their respective identification numbers. Slaughter techniques were in compliance with national regulations (FSIS, 2013).

Carcass Traits

After the slaughter, hot carcass weight (HCW) was recorded. The dressing percentage was calculated by the ratio of HCW and final live BW. Carcasses were chilling for 24 hr at 4°C, then, cold carcass weight (CCW) was acquired to calculate cooling loss through the ratio of CCW and HCW. Using a ruler, dorsal subcutaneous adipose tissue thickness was measured in millimeters at the first, tenth, and last ribs. Boneless loin was removed from the right side of each carcass, immediately identified and weighted. A 250 g loin section was removed from each loin for color and marbling evaluation, loin eye area calculation (LEA), loin chemical composition analysis, and drip loss determination. Carcass traits are presented in Table 3, 4, and 5.

Meat color was evaluated using a color panel scale of 1 to 6 according to National Pork Producers Council (1991) standards and objectively by the collection of lightness (l*), redness (a*), and yellowness (b*) values of cranial, medial, and caudal portion of each loin section (Model CR-410 Chromameter, Konica Minolta Sensing Inc., Osaka, Japan). The

marbling was subjective scored using a marbling scale of 1 to 10 according to National Pork Producers Council (1991) guideline. The surface of each loin eye section was drawn on a transparency paper and loin eye area (LEA) was measured with a grid paper in square centimeters. A 50 g portion of the each loin section was designated to chemical composition analysis to determine meat dry matter, ether extract, ash, and crude protein following AOAC (1990) procedures. A slice of about 3-cm of each loin eye section was weighed (W1), hanged into a sealed polyethylene plastic bag and placed into a 4°C refrigerator. After 48 hr, each slice was gently dried, weighed (W2) and the drip loss was calculated by the difference between W1 and W2 (Dribi et al., 2014).

Intestinal Sampling

Immediately after slaughter, the gastrointestinal tract of each animal was gently removed and identified. Proximal portion of jejunum and colon of each pig were collected and 7-cm sections were obtained. Each section was gently flushed and submerged into a 10% formaldehyde-phosphate buffer in an individually marked container for further microscopic morphology evaluation. The distal portion of jejunum and colon were also obtained to collect 1.5 mL of mucosal samples, which were immediately placed in liquid nitrogen for short period and transferred to -80°C freezer until TNFα measurement (Zhao et al., 2014).

Intestinal Morphology

The formalin-fixed jejunum and colon sections were trimmed and placed in cassettes. The North Carolina State University Histopathology Laboratory (College of Veterinary Medicine, Raleigh, NC) embedded the samples in paraffin, thin-sectioned and secured them on microscope slides with hematoxylin and eosin staining. The slides were evaluated under a

Sony CCD video camera attached to a microscope with 40x magnification. An average of 10 villus height, villus width and crypt depth were measured in each slide following the methodology described by Shen et al. (2009).

Mucosal Cytokine

The tumor necrosis factor alpha (TNFα) was also measured in jejunum and colon mucosal samples. A range of 0.50 - 0.51 g of each mucosa sample was homogenized with 1 mL of PBS solution, grounded and centrifuged for 10 min at 14,000 rpm. A 2 mL of supernatant was obtained for total protein calculation by BCA assay (Peace et al., 2011). After total protein measurement, TNFα was obtained by porcine TNFα colorimetric ELISA kit (Pierce Biotechnology, Inc., Rockford, IL). TNFα results were expressed in pg/mg of total protein (Table 6).

Statistical Analysis

This study used a randomized complete block design having sex and treatment as fixed effects and initial body weight blocks as a random effect. For growth performance (BW, ADG, ADFI, G: F) analysis, pen was considered the experimental unit. The remaining measurements had the harvested pigs (one per pen) as the experimental unit. All data were analyzed by Proc Mixed procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). A probability level of $P < 0.05$ was considered statistically significant whereas the probability of $0.05 \leq P < 0.10$ was considered trend.

Results

Growth Performance

Initial BW did not differ ($P > 0.10$) among treatments (Table 2). The dietary supplementation of DFM tended to increase ($P = 0.057$) ADG (by 33%, 0.73 to 0.97 kg/d) during the second week of phase 6 and tended to increase ($P = 0.062$) overall ADG (by 12%, 0.79 to 0.88).

The dietary supplementation of DFM tended to increase ($P = 0.072$) ADFI (by 23%, 1.8 to 2.25 kg/d) during the second week of phase 7 and increased ($P < 0.05$) the overall ADFI (by 9%, 2.56 to 2.79 kg/d). The feed efficiency (G:F) was not affected ($P > 0.10$).

Carcass Traits

Carcass traits are presented on Table 3. The supplementation of DFM did not affect ($P > 0.10$) cooling loss, backfat thicknesses, loin weight, and loin eye area. However, the supplementation of DFM increased ($P < 0.05$) hot carcass weight (by 9%, 85 to 92 kg), cold carcass weight (by 9%, 81 to 88 kg), and dressing percentage (by 2%, 77 to 78 %) of growing-finishing pigs.

Loin Composition

The loin nutrient composition is present on Table 4. The dietary supplementation of DFM did not affect ($P > 0.10$) the percentage of DM, ether extract, ash, and CP of growing-finishing pigs.

Meat Quality

The meat quality is present in Table 5. The dietary supplementation of DFM did not affect ($P > 0.10$) the drip loss (DL %), lightness (l*), redness (a*), yellowness (b*), and marbling (LM) of growing-finishing pigs.

Inflammatory Cytokine and Blood Urea Nitrogen

The tumor necrosis factor alpha and blood urea nitrogen values are present in Table 6. The dietary supplementation of DFM did not affect ($P > 0.10$) TNFα and blood urea nitrogen of growing-finishing pigs.

Intestinal Morphology Evaluation

The intestinal morphology measurements are present in Table 7. The dietary supplementation of DFM did not affect ($P > 0.10$) villus height, villus width, and crypt of growing-finishing pigs.

Discussion

The current study showed that the dietary supplementation of multi-strain DFM enhanced average daily feed intake. This result agreed and disagreed with other studies that utilized the same multi-strain DFM used in this current study. Davis and Anderson (2001) found that hens supplemented with the same multi-strain DFM used in this study increased growth performance. Chichlowski et al. (2007), Talebi et al. (2008), and Li et al. (2009) showed that the supplementation of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* ($1x10^8$ cfu/g) increased feed intake and weight gain of broilers and turkey. Due to limited literature related to the use of

this DFM in growing-finishing pigs, Maxwell et al. (1983) was the only study that reported weight gain and feed intake were not affected during growers and finishers phases.

Studies that used other sources of multi-strain DFM also showed variability regarding the benefits on growth performance. Choi et al. (2014) presented that a combination of *Lactobacillus acidophilus*, *Bacillus subtilis*, *Saccharomyces cerevisae*, and *Aspergillus oryzae* enhanced feed intake and average daily gain of weaning pigs on phase 2. Meng et al. (2014) found that *Bacillus subtilis* and *Clostridium butyricum* enhanced growth and feed efficiency of growing-finishing pigs but did not affect feed intake. Shon et al. (2005) and Kornegay and Risley (1996) did not observe growth enhancement of growing-finishing pigs fed a mixture of *Lactobacillus Reuteri*, *Lactobacillus salivarious*, and *Lactobacillus plantarum*, and *Bacillus subtilis* and *Bacillus licheniformis*, respectively.

Feed intake levels may affect positively or negatively the growth rate, feed efficiency, carcass quality, and consequently profitability of swine producers (Carroll and Allee, 2009). The gastrointestinal tract is considered the major neurological, immune, and endocrine organ, which communicates with the brain through vagal and spinal afferent neurons, gut hormones, and cytokines to regulate feeding behavior (Holzer et al., 2001; Wittig and Zeitz, 2003; Murphy and Bloom, 2006). Besides the beneficial effects of DFM in the small and large intestine microbiota and immunity, little is known about the effects of DFM on the gut hormones and peptides, such as neuropeptide Y, peptide YY, leptin, ghrelin, and galanin (Forsythe and Kunze, 2012). However, due to close relationship between gut microorganisms and intestinal endocrine cells, it is plausible to formulate a new hypothesis that multi-strain DFM could influence gut hormonal and peptide signaling, which may affect feeding behavior

Recently studies using mice have showed that a well-balanced gut microbiota communicates to the brain through biologically active gut peptides released from intestinal endocrine cells (Clarke et al., 2010; Peter et al., 2012). Bravo et al. (2011) showed that mice supplemented with *Lactobacillus rhammosus* for a long period had a significant impact on brain neurochemistry, reduce anxiety and depression behavior, and improve response against stress. Lesniewska et al. (2006) demonstrated that the supplementation of *Lactobacillus rhamnosus*, *Bifidobacterium lactis*, and inulin decreased the plasma levels of NPY and PPY in adult rats whereas did not affect concentration of PYY and decreased NPY levels in elderly rats, indicating that gut microbiota altered gut hormone release being the effect age dependent. Fetissov et al. (2008) identified that *Lactobacilli* has a homology protein sequence of ghrelin, PYY, NPY, and other regulatory peptides, which suggest that microbial proteins may be able to modulate the hormonal signaling pathways, and then influence appetite and emotional of animals supplemented with acid lactic bacteria. However, the mechanisms involved in gut hormonal and peptide response through DFM supplementation need further investigation once it was not the primarily goal of this current study.

In this study, the dietary supplementation of multi-strain DFM increased feed intake, and tended to enhance weight gain, consequently, increased dressing percentage of growing-finishing pigs. It suggests that multi-strain DFM may generate superior carcass yield due to possible stimulation of appetite behavior, followed by higher nutrient uptake, higher protein synthesis or lower protein degradation. The gut morphology and blood and urea nitrogen of pigs were the other variables investigated in this study once it may reflect the effect of multi-strain DFM over nutrient uptake and protein turnover, respectively.

The gut morphology was used to reveal gut health status (Choi et al., 2014). The villus height and crypt depth play an important rule on nutrient absorption (Fuller, 2004). Several studies reported that multi-strain DFM increased villus height in piglets (Mourao et al., 2005; Willing and Van Kessel, 2007; Di Giancamillo et al., 2008; Yang et al., 2009). Other studies showed that the supplementation of *Lactobacillus* enhanced villus height and crypt depth of weanling and growing-finishing pigs (Choi et al, 2014; Suo et al., 2012; Willing and Van Kessel, 2007). Yang et al. (2009) reported that crypt depth decreased in mice supplemented with moderate or high doses of DFM and increase with the supplementation of lower dose of the same DFM. In current study, villus height and crypt depth of pigs fed multi-strain DFM supplementation were not altered. It may be explained by the fact that the effect of DFM on villus height and crypt in older pigs may be restricted once they have more stable and mature gut microbiota (Konstantinov et al, 2006; Suo et al., 2012).

According to Bradford (2014), the blood urea nitrogen is a usual approach to measure the levels of amino acid oxidation and nitrogen excretion. Urea is an ammonia product, which is derived from amino acid catabolism mainly produced in the liver. When urea reaches the intestine, it is broken down by urease, which is released by gut microbiota, and then, the nitrogen is recycled to the liver or excreted. In this study, BUN did not differ between the treatments indicating that the inclusion of DFM did not affect nitrogen digestibility.

Therefore, once multi-strain DFM supplementation increased feed intake and carcass yield, and tended to increase weight gain without affecting loin quality, intestinal morphology, and blood urea nitrogen profile, further investigation is needed to explain a

possible relationship between DFM microorganisms with gut hormones and gut peptides, which regulates appetite behavior and protein turnover of growing-finishing pigs.

Conclusion

Collectively, the supplementation of 0.05% of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* ($1x10^8$ cfu/g) enhanced feed intake, hot carcass weight, cold carcass weight, and dressing percentage and tended to enhance weight gain of growing-finishing pigs without affecting serum and mucosa cytokine levels, blood and urea nitrogen, and meat quality of growing-finishing pigs. According to this study outcome, multi-strain DFM may influence gut hormones and peptides responsible for appetite regulation and play an interesting rule over protein turnover. However, further research is required to explain this new hypothesis.

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Table 1. Composition of DFM and CON diets (Phase 6: d 0 to 14 and Phase 7: d 14 to 28)

	Phase 6		Phase 7	
Item	**CON**	**DFM[1]**	**CON**	**DFM[1]**
Ingredient, %				
Yellow corn	74.15	74.15	77.92	77.92
Soybean meal	12.74	12.69	9.2	9.15
DDGS	10	10	10	10
L-Lys HCl	0.26	0.26	0.22	0.22
L-Thr	0.03	0.03	0.02	0.02
Salt	0.22	0.22	0.22	0.22
Vitamin premix[2]	0.03	0.03	0.03	0.03
Mineral premix[3]	0.15	0.15	0.15	0.15
Dical P (26Ca18P)	0.5	0.5	0.4	0.4
Limestone	0.92	0.92	0.84	0.84
Poultry fat	1	1	1	1
DFM[1]	0	0.05	0	0.05
Calculated composition				
DM, %	88.99	88.99	88.9	88.9
ME, Mcal/kg	3.370	3.370	3.379	3.379
CP, %	15.19	15.19	13.77	13.77
Lys, %	0.73	0.73	0.61	0.61
Cys + Met, %	0.46	0.46	0.43	0.43
Trp, %	0.13	0.13	0.11	0.11
Thr, %	0.46	0.46	0.40	0.40
Ca, %	0.52	0.52	0.46	0.46
Available P, %	0.24	0.24	0.21	0.21
Total P, %	0.44	0.44	0.4	0.4
Analyzed composition				
DM, %	90.57	90.82	90.47	90.50
CP, %	14.09	14.02	13.13	13.88
ADF, %	4.25	4.43	3.24	3.38
Ca, %	0.60	0.56	0.51	0.53
P, %	0.45	0.44	0.41	0.43

Table 1. Continued

[1]: A mixture of *Lactobactillus acidophilus* (2.5 x 10^7cfu/gram), *Lactobacillus casei* (2.5 x 10^7cfu/gram), *Bifidobacterium thermophilum* (2.5 x 10^7cfu/gram) and *Enterococcus faecium* (2.5 x 10^7cfu/gram) with a concentration of 1 x 10^8 cfu/gram.

[2]:Vitamin premix provided the following per kilogram of complete diet: 22,045,000 IU of vitamin A; 3,306,900 IU of vitamin D_3; 66,138 IU of vitamin K; 88 mg of vitamin B_{12}; 15,432 mg of riboflavin; 88,184 mg of niacin; 61,729 mg of d-pantothenic acid; 220 mg of biotin.

[3]: Mineral premix provided the following composition: 1.10% of Cu; 198.0 mg/kg of I; 11.02% of Fe; 2.64% of Mn; 198.4 mg/kg of Se; 11.02% of Zn.

Table 2. Growth performance of growing-finishing pigs fed diets supplemented with DFM.

Item	Treatment		SEM	*P* value
	CON	DFM[1]		
BW, kg				
Initial	88.5	88.5	0.6	0.910
Wk 1 (Phase 6)	93.2	93.7	0.9	0.707
Wk 2 (Phase 6)	98.3	100.6	1.0	0.153
Wk 1 (Phase 7)	103.6	105.8	1.2	0.173
Wk 2 (Phase 7)	110.7	113.4	1.1	0.119
ADG, Kg/d				
Wk 1 (Phase 6)	0.671	0.753	0.103	0.580
Wk 2 (Phase 6)	0.732	0.976	0.084	0.057
Wk 1 (Phase 7)	0.748	0.742	0.064	0.936
Wk 2 (Phase 7)	1.011	1.084	0.089	0.566
Overall	0.791	0.889	0.034	0.062
ADFI, kg/d				
Wk 1 (Phase 6)	2.287	2.563	0.154	0.224
Wk 2 (Phase 6)	2.637	2.895	0.115	0.135
Wk 1 (Phase 7)	3.491	3.447	0.126	0.810
Wk 2 (Phase 7)	1.828	2.255	0.155	0.072
Overall	2.561	2.790	0.067	0.029
G:F				
Wk 1 (Phase 6)	0.282	0.284	0.035	0.981
Wk 2 (Phase 6)	0.27	0.343	0.032	0.129
Wk 1 (Phase 7)	0.214	0.215	0.018	0.930
Wk 2 (Phase 7)	0.572	0.486	0.056	0.298
Overall	0.380	0.384	0.059	0.955

[1]: A mixture of *Lactobactillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus* with a concentration of $1x10^8$ cfu/gram.

Table 3. Hot carcass weight (HCW), cold carcass weight (CCW), cooling loss (CL), dressing (DRESS), loin eye area (LEA), loin weight (LW), backfat thickness at 1st rib (BTx1), backfat thickness at 10th rib (BTx10), and backfat thickness at last rib (BT x LR) of growing-finishing pigs fed DFM.

	Treatment			***P* value**
Item	**CON**	**DFM[1]**	**SEM**	
Final BW (kg)	110.4	117.5	1.88	0.018
HCW (kg)	85.32	92.75	1.74	0.009
CCW (kg)	81.73	88.94	1.7	0.011
CL (%)	4.2	4.11	0.29	0.758
DRESS (%)	77.32	78.88	0.41	0.035
LW (kg)	6.64	7.55	0.37	0.108
LEA (cm^2)	54.4	53.8	3.24	0.897
BTx1 (mm)	27.8	29.9	1.29	0.27
BTx10 (mm)	20.2	22.7	1.17	0.156
BTxLR(mm)	25.6	25.9	1.48	0.889

[1]: A mixture of *Lactobactillus acidophilus*, *Lactobacillus case*i, *Bifidobacterium thermophilum*, and *Enterococcus faecium* with a concentration of $1x10^8$ cfu/gram.

Table 4. Centesimal composition of *Longissimus dorsi* muscle of growing-finishing pigs fed DFM.

	Treatment			
Item	**CON**	**DFM[1]**	**SEM**	***P* value**
DM	28.40	28.00	0.59	0.573
Fat DM Basis%	16.30	14.60	2.31	0.610
Fat As-is%	4.70	4.20	0.78	0.609
Ash DM Basis%	3.70	3.90	0.17	0.376
Ash as-is%	1.04	1.08	0.03	0.309
CP DM Basis%	76.89	80.41	1.78	0.184
CP As-is%	21.75	22.45	0.32	0.150

[1]: A mixture of *Lactobactillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* with a concentration of $1x10^8$ cfu/gram.

Table 5. Drip loss (DL), lightness (L*), redness (A*), yellowness (B*) and loin marbling (LM) of *Longissimus dorsi* muscle of growing-finishing pigs fed DFM.

	Treatment			
Item	**CON**	**DFM[1]**	**SEM**	***P* value**
DL %	4.63	5.88	0.52	0.117
L*	51.38	52.98	0.67	0.116
A*	6.43	6.34	0.38	0.816
B*	4.52	4.90	0.22	0.252
LM	2.40	2.90	0.27	0.214

[1]: A mixture of *Lactobactillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* with a concentration of $1x10^8$ cfu/gram.

Table 6. TNFα blood, TNFα jejunum, TNFα colon and BUN of growing-finishing pigs fed DFM.

	Treatment			
Item	**CON**	**DFM[1]**	**SEM**	***P* value**
TNFα Blood (pg/mL)	84.05	73.38	7.42	0.327
TNFα Jejunum (pg/mg protein)	0.33	0.26	0.06	0.468
TNFα Colon (pg/mg of protein)	1.45	1.67	0.32	0.556
BUN (mg/dL)	9.27	7.59	1.25	0.204

[1]: A mixture of *Lactobactillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium*, and *Enterococcus faecium* with a concentration of 1 x 10^8 cfu/gram.

Table 7. Villus height, width, crypt depth and villus height to crypt depth ratio from jejunum and colon of growing-finishing pigs fed DFM.

	Treatment			
Item	**Control**	**DFM[1]**	**SEM**	***P* value**
Jejunum (µm)				
Villus height	325.27	316.84	20.67	0.538
Villus width	132.12	133.64	5.65	0.852
Crypt depth	217.88	215.21	12.02	0.869
VCR[3]	1.562	1.47	0.07	0.424
Colon (µm)				
Crypt depth	355.83	310.86	19.07	0.117

[1]: A mixture of *Lactobactillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* with a concentration of 1 x 10^8 cfu/gram.
[3]: Villus height to crypt depth ratio.

CHAPTER 4

GENERAL CONCLUSION

The growth performance and carcass traits of growing-finishing pigs are directly associated with producers profitability. The use of low doses of antimicrobials and growth-promoter factors as feed additives are a common practice in the United States livestock industry. The new antimicrobial regulation and the banned or restriction of mainly growth-promoter factors have been stimulating livestock producers to find alternatives to maintain high productivity levels and production costs. Mono-strain and multi-strain direct-fed microbials might be a strategy. The DFM is a source of live and viable microorganisms, which colonize the small and large intestine beneficiating the overall health status of pigs, poultry, beef cattle, dairy cows, horses, and humans. It plays an important role against pathogenic invasion, enhances immunity, increases intestinal microbiota balance, and consequently, may optimize growth performance and carcass traits of farm animals.

Direct-fed microbial efficacy depends on several factors, which may explain the variability in results. The DFM strain specificity, stability, and dosage are crucial factors for an optimal response, however, animal health condition, nutrition status, age, stress, and genetic are also fundamental to achieve a desirable DFM response. The dietary supplementation of mono-strain DFM based on *Lactobacillus acidophilus* did not affect growth performance and loin quality of growing-finishing pigs, but reduced backfack thickness. Further, the dietary inclusion of multi-strain DFM based on *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* tended to enhance weight gain and improved carcass yield without affecting loin quality.

For the mono-strain study, further investigations are required to clarify a possible association between *Lactobacillus* and lipolytic effects. For the multi-strain study, further

analyzes are needed to explain in more details the DFM influence on gut hormones and gut peptides, which are responsible to regulate appetite behavior.

APPENDICES

Appendix A

Table 1. Weekly body weight of growing-finishing pigs fed diets supplemented with mono-strain DFM.

Sex	Trt	Block	Phase 5					Phase 6			Phase 7		
			Initial BW	BW1	BW2	BW3	BW4	BW5	BW6	BW7	BW8	BW9	BW10
m	DFM	1	118.4	137.2	156.2	175	191.8	205.4	228.6	240.2	254.4	274.8	289
m	DFM	1	116.8	129.8	152	175.2	189.6	181.6	210.8	221.6	234.6	248.8	267.2
m	DFM	1	111.6	132.2	152.8	174.8	189.6	200	223.6	230.6	248.2	271.8	288.6
m	CON	1	120	140.8	160.6	180.2	192.4	211	226.6	235.2	251.6	270.8	289.2
m	CON	1	117.4	134.8	157	181.6	196.6	211.6	223.2	231.8	239.2	261.6	278.2
m	CON	1	112.2	133.2	152.8	180.8	193.2	208.6	229.2	245.6	267	290.2	311.2
f	CON	6	113.5	129.2	132.4	157.6	170.2	191	204.2	210.6	221.2	236.6	259.6
f	CON	6	111.4	132.2	150.4	171.6	181.4	199.6	207.6	220.2	240.6	257	273
f	CON	6	109.6	130.6	143.2	160.8	169.6	190.2	189.8	200.4	212.6	230.6	250.2
f	DFM	6	113.4	131	150.6	172.8	184	200.4	216.4	225.2	237.8	252.1	268.2
f	DFM	6	111.8	127.6	143.6	164.2	179.2	193	210	214.2	220.8	236.4	259
f	DFM	6	110.6	124.6	144	164.8	175.2	189.2	207.6	217.4	229.2	246	265.5
m	CON	2	108.6	115	128.6	152.6	164.4	179.6	186.6	195.2	199.6	212	225.2
m	CON	2	107.4	122.8	141.8	160.2	173.6	192.8	202.4	217.6	232.7	252.8	273.6
m	CON	2	106.6	125.2	144.8	169	181	198.4	214.4	231.2	244.2	264.8	285.6
m	DFM	2	107.8	124	151.6	174.2	190	208.2	223.2	230.2	250.8	268.6	296
m	DFM	2	106.6	122.4	138.6	158.2	169.2	182.6	185.6	198.6	204.8	219.6	239
m	DFM	2	106.2	123.4	141.6	160.2	169.8	190.2	201.4	215.6	232	246.8	265.4
f	CON	7	107.6	125.2	146.4	160	166.8	183.2	195	210.6	219.4	240.8	253.6
f	CON	7	107.6	119	137.8	157.8	170.6	186.6	202.4	215.4	207.2	221.8	237.6
f	CON	7	106	117.8	137.4	158.2	171.4	190.2	205.6	220.8	229.4	246	266.4

Table 1. Continued

f	DFM	7	108.4	123.6	142.6	160.8	172.8	189	203.6	208.6	211.6	230.2	246.2
f	DFM	7	107.4	126.2	145.4	168.2	182.2	194.2	210.4	217.8	235.2	254.2	265.8
f	DFM	7	106.4	126.6	141.6	164.8	175.2	186.6	201.6	210.8	215.8	232	239.8
m	DFM	3	104.6	127.4	139.6	162.4	172.6	191.6	199	216	228.4	249.6	272.6
m	DFM	3	98.2	111	130	148.4	159.8	178.2	190	206.6	216.8	232.2	246
m	DFM	3	94.4	103.8	124.2	145.6	159.8	176.2	182.8	194	201.8	215.6	232.8
m	CON	3	103	118.4	139.6	163.6	182.4	198.2	204.4	219	228.4	249.6	270.4
m	CON	3	96.4	111.4	129.8	149	162.2	180.2	193.6	207.4	221.2	236.8	253.8
m	CON	3	94.2	106.8	123.6	144.4	157.8	175.6	189.4	206.8	222.4	241.4	258.8
f	CON	8	104	120.8	139.6	162.4	177.8	195.4	204.6	219.4	234.2	256.8	274.8
f	CON	8	102	119.2	138.6	158.8	174.4	189.4	205.6	215.2	225.8	244.2	261.2
f	CON	8	96.2	111	128.8	147.8	160	175.4	192.6	205.6	219.6	233.6	248.6
f	DFM	8	105.2	117.2	133.6	154	164.4	175.4	195.4	198.6	202.4	210.8	220.6
f	DFM	8	102.4	127	145.4	165.2	179.6	198.6	211.4	194.2	235	253.8	259.6
f	DFM	8	95.2	109	123.6	141.8	156.2	171.6	186.6	224.6	204.4	218.4	228
m	DFM	4	93.4	106.2	123.4	141.6	148.6	157.6	167.8	184.2	201.2	217.4	235.2
m	DFM	4	91.4	109.4	128.2	148.8	162.6	172	183.6	196.4	211.6	230.6	245.4
m	DFM	4	88.2	105.2	125.6	145	158.2	170.8	180.6	198.2	212.8	228.4	234.8
m	CON	4	92.4	104.8	120.8	139	153.2	160.4	168.4	179.4	193.6	201.8	222.8
m	CON	4	89.4	102.2	119.4	142.4	159.4	172.2	187.2	203.4	221	240.6	259.8
m	CON	4	86	98.8	116.6	138.4	152	169.6	182.6	199.4	216.6	236.4	252
f	CON	9	95.2	110	120	137	146	163.4	169.4	184.6	194.4	210.2	222.4
f	CON	9	90.4	109	127	146.8	159.8	177	189.4	205.2	213.6	231.4	231.4
f	CON	9	86	97.6	112.2	133.2	146.6	156.6	162.8	171.8	183.2	191.6	202.2

Table 1. Continued

f	DFM	9	93.8	104	117	137.8	148.4	161.2	170.6	176.2	187.2	200.2	212.8
f	DFM	9	90.2	106.4	121.4	139	151.4	165.6	176	191.6	208.4	224	235.6
f	DFM	9	85.8	100.2	113.6	129.6	141.2	151.8	158.6	169.4	181.8	193.4	208
m	CON	5	85	105.2	123.6	146.2	156.4	174.2	180.2	188.2	206.2	226.4	244.
m	CON	5	79.4	99.2	116.2	139	152.8	167.6	178.4	186.2	204.6	230.4	252.8
m	CON	5	71.6	86.4	101	120.6	132.2	147.2	164.6	175.6	185.6	204.2	218.2
m	DFM	5	81.6	94.2	108	128	137.6	148.6	155.2	166.4	180.6	199.2	216.6
m	DFM	5	78.6	97	113.6	136.6	147.8	166.4	178.4	196.4	213.4	232.8	251.4
m	DFM	5	73.6	88.6	104.6	126.4	139.2	155	164.6	182.4	199.4	218.4	236.4
f	DFM	10	84	93.4	107.6	127	141	154.4	174.5	177.6	188.6	203.8	215.6
f	DFM	10	77.6	90.2	105.4	125.4	142.2	157.6	178	180.8	186.6	205.2	219.6
f	DFM	10	74.4	90.6	103.4	122.2	135.6	149	167	170	170.6	185.2	196.2
f	CON	10	82.8	95.8	113.4	132.4	142.8	152.6	165.6	171.2	184.6	203.2	213.8
f	CON	10	81.6	99.6	117	134.4	147.8	162.2	167.6	174.8	185.2	197.4	213
f	CON	10	74.8	89	104.4	126.4	139.6	153.4	154.4	165.8	173.6	186.4	193.8

[1]: Lactobacillus acidophilus $1x10^9$ cfu/gram.

Table 2. Weekly ADG of growing-finishing pigs fed diets supplemented with mono-strain DFM.

Sex	Trt	Block	Phase 5				Phase 6				Phase 7	
			ADGW1	ADGW2	ADGW3	ADG W4	ADG W5	ADG W6	ADG W7	ADG W8	ADG W9	ADG W10
m	DFM	1	2.69	2.71	2.69	2.40	1.94	3.31	1.66	2.03	2.91	2.03
m	DFM	1	1.86	3.17	3.31	2.06	-1.14	4.17	1.54	1.86	2.03	2.63
m	DFM	1	2.94	2.94	3.14	2.11	1.49	3.37	1.00	2.51	3.37	2.40
m	CON	1	2.97	2.83	2.80	1.74	2.66	2.23	1.23	2.34	2.74	2.63
m	CON	1	2.49	3.17	3.51	2.14	2.14	1.66	1.23	1.06	3.20	2.37
m	CON	1	3.00	2.80	4.00	1.77	2.20	2.94	2.34	3.06	3.31	3.00
f	CON	6	2.24	0.46	3.60	1.80	2.97	1.89	0.91	1.51	2.20	3.29
f	CON	6	2.97	2.60	3.03	1.40	2.60	1.14	1.80	2.91	2.34	2.29
f	CON	6	3.00	1.80	2.51	1.26	2.94	-0.06	1.51	1.74	2.57	2.80
f	DFM	6	2.51	2.80	3.17	1.60	2.34	2.29	1.26	1.80	2.04	2.30
f	DFM	6	2.26	2.29	2.94	2.14	1.97	2.43	0.60	0.94	2.23	3.23
f	DFM	6	2.00	2.77	2.97	1.49	2.00	2.63	1.40	1.69	2.40	2.79
m	CON	2	0.91	1.94	3.43	1.69	2.17	1.00	1.23	0.63	1.77	1.89
m	CON	2	2.20	2.71	2.63	1.91	2.74	1.37	2.17	2.16	2.87	2.97
m	CON	2	2.66	2.80	3.46	1.71	2.49	2.29	2.40	1.86	2.94	2.97
m	DFM	2	2.31	3.94	3.23	2.26	2.60	2.14	1.00	2.94	2.54	3.91
m	DFM	2	2.26	2.31	2.80	1.57	1.91	0.43	1.86	0.89	2.11	2.77
m	DFM	2	2.46	2.60	2.66	1.37	2.91	1.60	2.03	2.34	2.11	2.66
f	CON	7	2.51	3.03	1.94	0.97	2.34	1.69	2.23	1.26	3.06	1.83
f	CON	7	1.63	2.69	2.86	1.83	2.29	2.26	1.86	-1.17	2.09	2.26
f	CON	7	1.69	2.80	2.97	1.89	2.69	2.20	2.17	1.23	2.37	2.91
f	DFM	7	2.17	2.71	2.60	1.71	2.31	2.09	0.71	0.43	2.66	2.29
f	DFM	7	2.69	2.74	3.26	2.00	1.71	2.31	1.06	2.49	2.71	1.66

Table 2. Continued

f	DFM	7	2.89	2.14	3.31	1.49	1.63	2.14	1.31	0.71	2.31	1.11
m	DFM	3	3.26	1.74	3.26	1.46	2.71	1.06	2.43	1.77	3.03	3.29
m	DFM	3	1.83	2.71	2.63	1.63	2.63	1.69	2.37	1.46	2.20	1.97
m	DFM	3	1.34	2.91	3.06	2.03	2.34	0.94	1.60	1.11	1.97	2.46
m	CON	3	2.20	3.03	3.43	2.69	2.26	0.89	2.09	1.34	3.03	2.97
m	CON	3	2.14	2.63	2.74	1.89	2.57	1.91	1.97	1.97	2.23	2.43
m	CON	3	1.80	2.40	2.97	1.91	2.54	1.97	2.49	2.23	2.71	2.49
f	CON	8	2.40	2.69	3.26	2.20	2.51	1.31	2.11	2.11	3.23	2.57
f	CON	8	2.46	2.77	2.89	2.23	2.14	2.31	1.37	1.51	2.63	2.43
f	CON	8	2.11	2.54	2.71	1.74	2.20	2.46	1.86	2.00	2.00	2.14
f	DFM	8	1.71	2.34	2.91	1.49	1.57	2.86	0.46	0.54	1.20	1.40
f	DFM	8	3.51	2.63	2.83	2.06	2.71	1.83	-2.46	5.83	2.69	0.83
f	DFM	8	1.97	2.09	2.60	2.06	2.20	2.14	5.43	-2.89	2.00	1.37
m	DFM	4	1.83	2.46	2.60	1.00	1.29	1.46	2.34	2.43	2.31	2.54
m	DFM	4	2.57	2.69	2.94	1.97	1.34	1.66	1.83	2.17	2.71	2.11
m	DFM	4	2.43	2.91	2.77	1.89	1.80	1.40	2.51	2.09	2.23	0.91
m	CON	4	1.77	2.29	2.60	2.03	1.03	1.14	1.57	2.03	1.17	3.00
m	CON	4	1.83	2.46	3.29	2.43	1.83	2.14	2.31	2.51	2.80	2.74
m	CON	4	1.83	2.54	3.11	1.94	2.51	1.86	2.40	2.46	2.83	2.23
f	CON	9	2.11	1.43	2.43	1.29	2.49	0.86	2.17	1.40	2.26	1.74
f	CON	9	2.66	2.57	2.83	1.86	2.46	1.77	2.26	1.20	2.54	0.00
f	CON	9	1.66	2.09	3.00	1.91	1.43	0.89	1.29	1.63	1.20	1.51
f	DFM	9	1.46	1.86	2.97	1.51	1.83	1.34	0.80	1.57	1.86	1.80
f	DFM	9	2.31	2.14	2.51	1.77	2.03	1.49	2.23	2.40	2.23	1.66
f	DFM	9	2.06	1.91	2.29	1.66	1.51	0.97	1.54	1.77	1.66	2.09

Table 2. Continued

m	CON	5	2.89	2.63	3.23	1.46	2.54	0.86	1.14	2.57	2.89	2.54
m	CON	5	2.83	2.43	3.26	1.97	2.11	1.54	1.11	2.63	3.69	3.20
m	CON	5	2.11	2.09	2.80	1.66	2.14	2.49	1.57	1.43	2.66	2.00
m	DFM	5	1.80	1.97	2.86	1.37	1.57	0.94	1.60	2.03	2.66	2.49
m	DFM	5	2.63	2.37	3.29	1.60	2.66	1.71	2.57	2.43	2.77	2.66
m	DFM	5	2.14	2.29	3.11	1.83	2.26	1.37	2.54	2.43	2.71	2.57
f	DFM	10	1.34	2.03	2.77	2.00	1.91	2.87	0.44	1.57	2.17	1.69
f	DFM	10	1.80	2.17	2.86	2.40	2.20	2.91	0.40	0.83	2.66	2.06
f	DFM	10	2.31	1.83	2.69	1.91	1.91	2.57	0.43	0.09	2.09	1.57
f	CON	10	1.86	2.51	2.71	1.49	1.40	1.86	0.80	1.91	2.66	1.51
f	CON	10	2.57	2.49	2.49	1.91	2.06	0.77	1.03	1.49	1.74	2.23
f	CON	10	2.03	2.20	3.14	1.89	1.97	0.14	1.63	1.11	1.83	1.06

[1]: Lactobacillus acidophilus $1x10^9$ cfu/gram.

Table 3. Weekly body weight of growing-finishing pigs fed diets supplemented with multi-strain DFM.

Sex	Trt	Block		Phase 6		Phase 7	
			Initial BW	BW1	BW2	BW3	BW4
f	PC	1	216	227.6	241.8	247.2	270.2
f	PC	1	173.4	179.6	198.4	212.6	229
f	PC	1	189.4	201.2	217.4	228.4	251.6
f	PC	1	187.2	180.6	186.4	202.6	217.6
f	PC	1	214.2	225.2	241.8	255.2	267.8
f	PC	1	178.8	180.2	196.4	206	228.8
f	PT	1	213.2	218	234.4	243.4	254
f	PT	1	206.6	219.2	233.2	237.4	255.8
f	PT	1	167.8	181.4	195.6	205	207.2
f	PT	1	202	216.6	236.4	246.4	263.8
f	PT	1	176.4	186	196.4	208.8	228.6
f	PT	1	213.6	227.2	242.6	254.8	273.8
f	PC	2	200.2	213.2	224.8	244.2	267.6
f	PC	2	208.8	213.4	234.2	248.6	264.8
f	PC	2	185.2	193.2	210.8	222.6	238
f	PT	2	207.6	216.6	234	249.4	264.6
f	PT	2	177.2	187.4	207.2	217.6	238.4
f	PT	2	195.6	204.6	217.4	231	246.8
f	PT	2	199.2	217.8	228.4	235.6	254.6
f	PT	2	183.2	190.4	211.4	221.6	236.2
f	PT	2	205.4	221.6	228.6	235.6	254.8
f	PC	2	206.2	223.6	223.8	238.2	237.4
f	PC	2	207.6	228.2	244.8	256	261
f	PC	2	188.2	202.3	218.2	227.8	231.8
m	PT	3	217.2	233	249.6	261.6	279
m	PT	3	204.4	225.2	237.2	249.6	266.8
m	PT	3	161.6	177.6	185.6	199	214.4
m	PT	3	201.2	215	233.6	236.6	250.4
m	PT	3	174.8	190.6	210.2	223.8	240.8
m	PT	3	215.6	231	239.2	257.2	281.6
m	PC	3	163.4	168.4	176	181.6	197.4
m	PC	3	219.8	225.2	235.6	250.2	275
m	PC	3	190	198.6	209.8	215.6	235
m	PC	3	188.8	195.2	202.6	221.6	239.8
m	PC	3	218.6	225.4	.	.	.

Table 3. Continued

m	PC	3	175.4	180.4	194	203.8	219
m	PT	4	208.6	209.2	217.4	232.4	252.6
m	PT	4	185.8	187.6	209	229.6	248.6
m	PT	4	193.2	200.6	217	232.8	253.6
m	PC	4	214.6	227.8	240.8	253.8	274.2
m	PC	4	204.2	220.4	.	.	.
m	PC	4	185	186.6	211.2	228.4	244
m	PC	4	200.6	220.8	228.4	244.4	262
m	PC	4	209	209.6	230	235.6	244.6
m	PC	4	186.8	200	202.6	222.2	238
m	PT	4	199.2	222.6	242.6	251.4	259.4
m	PT	4	183.8	191.8	192.6	208.8	220.4
m	PT	4	208.6	223.6	242.2	250	264.8
f	PC	5	190.2	203.2	212.8	217.6	237.8
f	PC	5	180	199.8	205.6	218.6	228.6
f	PC	5	206.6	224.6	220.6	236.6	254.8
f	PC	5	204.4	221.8	236.4	234.8	253.6
f	PC	5	188	200.2	220.6	225	226.9
f	PC	5	174.2	189.2	205	217.8	234
m	PT	5	180.2	202.2	217.6	227.6	249.2
m	PT	5	205.4	218.8	237.4	248.6	266.8
m	PT	5	192.2	203.4	217	229.8	248.2
m	PT	5	202.6	207	222.8	234.4	250.6
m	PT	5	178.6	185.2	203.8	212.4	229.6
m	PT	5	190.2	188.6	211	222.6	241.4

[1]: A mixture of *Lactobactillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* with a concentration of $1x10^8$ cfu/gram.

Table 4. Weekly ADG of growing-finishing pigs fed diets supplemented with multi-strain DFM.

Sex	Trt	Block	Phase 6		Phase 7	
			ADG W1	ADG W2	ADG W3	ADG W4
f	PC	1	1.66	2.03	0.77	3.29
f	PC	1	0.89	2.69	2.03	2.34
f	PC	1	1.69	2.31	1.57	3.31
f	PC	1	-0.94	0.83	2.31	2.14
f	PC	1	1.57	2.37	1.91	1.80
f	PC	1	0.20	2.31	1.37	3.26
f	PT	1	0.69	2.34	1.29	1.51
f	PT	1	1.80	2.00	0.60	2.63
f	PT	1	1.94	2.03	1.34	0.31
f	PT	1	2.09	2.83	1.43	2.49
f	PT	1	1.37	1.49	1.77	2.83
f	PT	1	1.94	2.20	1.74	2.71
f	PC	2	1.86	1.66	2.77	3.34
f	PC	2	0.66	2.97	2.06	2.31
f	PC	2	1.14	2.51	1.69	2.20
f	PT	2	1.29	2.49	2.20	2.17
f	PT	2	1.46	2.83	1.49	2.97
f	PT	2	1.29	1.83	1.94	2.26
f	PT	2	2.66	1.51	1.03	2.71
f	PT	2	1.03	3.00	1.46	2.09
f	PT	2	2.31	1.00	1.00	2.74
f	PC	2	2.49	0.03	2.06	-0.11
f	PC	2	2.94	2.37	1.60	0.71
f	PC	2	2.01	2.27	1.37	0.57
m	PT	3	2.26	2.37	1.71	2.49
m	PT	3	2.97	1.71	1.77	2.46
m	PT	3	2.29	1.14	1.91	2.20
m	PT	3	1.97	2.66	0.43	1.97
m	PT	3	2.26	2.80	1.94	2.43
m	PT	3	2.20	1.17	2.57	3.49
m	PC	3	0.71	1.09	0.80	2.26
m	PC	3	0.77	1.49	2.09	3.54
m	PC	3	1.23	1.60	0.83	2.77
m	PC	3	0.91	1.06	2.71	2.60
m	PC	3	0.97	.	.	.

Table 4. Continued

m	PC	3	0.71	1.94	1.40	2.17
m	PT	4	0.09	1.17	2.14	2.89
m	PT	4	0.26	3.06	2.94	2.71
m	PT	4	1.06	2.34	2.26	2.97
m	PC	4	1.89	1.86	1.86	2.91
m	PC	4	2.31	.	.	.
m	PC	4	0.23	3.51	2.46	2.23
m	PC	4	2.89	1.09	2.29	2.51
m	PC	4	0.09	2.91	0.80	1.29
m	PC	4	1.89	0.37	2.80	2.26
m	PT	4	3.34	2.86	1.26	1.14
m	PT	4	1.14	0.11	2.31	1.66
m	PT	4	2.14	2.66	1.11	2.11
f	PC	5	1.86	1.37	0.69	2.89
f	PC	5	2.83	0.83	1.86	1.43
f	PC	5	2.57	-0.57	2.29	2.60
f	PC	5	2.49	2.09	-0.23	2.69
f	PC	5	1.74	2.91	0.63	0.27
f	PC	5	2.14	2.26	1.83	2.31
m	PT	5	3.14	2.20	1.43	3.09
m	PT	5	1.91	2.66	1.60	2.60
m	PT	5	1.60	1.94	1.83	2.63
m	PT	5	0.63	2.26	1.66	2.31
m	PT	5	0.94	2.66	1.23	2.46
m	PT	5	-0.23	3.20	1.66	2.69

[1]: A mixture of *Lactobactillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* with a concentration of $1x10^8$ cfu/gram.

Table 5. Temperature variations in Fahrenheit of the barn of growing-finishing fed diets supplemented with mono-strain DFM.

Phase	Date	Time	Current Temperature (ºF)	High Temperature (ºF)	Low Temperature (ºF)
Phase 5	5/15/2014	1:00 PM	78	86	70
Phase 5	5/16/2014	12:00 PM	71	79	61
Phase 5	5/17/2014	10:00 AM	64	73	59
Phase 5	5/18/2014	11:00 AM	66	71	63
Phase 5	5/19/2014	1:00 PM	72	73	62
Phase 5	5/20/2014	10:00 AM	70	73	62
Phase 5	5/21/2014	1:00 PM	84	84	64
Phase 5	5/22/2014	1:00 PM	85	86	70
Phase 5	5/23/2014	1:00 PM	84	88	73
Phase 5	5/24/2014	11:00 AM	74	86	67
Phase 5	5/25/2014	12:00 PM	80	81	70
Phase 5	5/26/2014	1:00 PM	82	83	70
Phase 5	5/27/2014	1:00 PM	86	86	70
Phase 5	5/28/2014	1:00 PM	86	89	70
Phase 5	5/29/2014	1:00 PM	75	85	70
Phase 5	5/30/2014	9:00 AM	70	79	66
Phase 5	5/31/2014	4:00 PM	80	81	70
Phase 5	6/1/2014	10:00 AM	69	81	62
Phase 5	6/2/2014	11:00 AM	72	79	62
Phase 5	6/3/2014	1:00 PM	83	83	63
Phase 5	6/4/2014	1:00 PM	88	89	70
Phase 5	6/5/2014	1:00 PM	88	88	76
Phase 5	6/6/2014	1:00 PM	80	88	72
Phase 5	6/7/2014	10:00 AM	75	80	70
Phase 5	6/8/2014	6:00 AM	80	86	70
Phase 5	6/9/2014	1:00 PM	83	83	71
Phase 5	6/10/2014	1:00 PM	88	89	72
Phase 5	6/11/2014	3:00 PM	88	92	78
Phase 6	6/12/2014	1:00 PM	78	91	70
Phase 6	6/13/2014	1:00 PM	82	82	71
Phase 6	6/14/2014	1:00 PM	83	86	70
Phase 6	6/15/2014	10:00 AM	74	85	69
Phase 6	6/16/2014	1:00 PM	88	89	78
Phase 6	6/17/2014	1:00 PM	90	92	78
Phase 6	6/18/2014	1:00 PM	90	94	78
Phase 6	6/19/2014	1:00 PM	92	93	78

Table 5. Continued

Phase 6	6/20/2014	1:00 PM	90	92	78
Phase 6	6/21/2014	11:00 AM	80	90	78
Phase 6	6/22/2014	10:00 AM	75	86	72
Phase 6	6/23/2014	1:00 PM	80	82	70
Phase 6	6/24/2014	1:00 PM	84	85	70
Phase 6	6/25/2014	1:00 PM	87	87	76
Phase 6	6/26/2014	1:00 PM	89	89	76
Phase 6	6/27/2014	1:00 PM	84	87	76
Phase 6	6/28/2014	12:00 PM	79	88	76
Phase 6	6/29/2014	10:00 AM	73	83	70
Phase 6	6/30/2014	2:00 PM	89	89	70
Phase 6	7/1/2014	1:00 PM	84	87	76
Phase 6	7/2/2014	1:00 PM	89	90	78
Phase 6	7/3/2014	2:00 PM	82	92	82
Phase 6	7/4/2014	2:00 PM	82	86	70
Phase 6	7/5/2014	1:00 PM	80	86	70
Phase 6	7/6/2014	11:00 AM	80	86	70
Phase 6	7/7/2014	1:00 PM	85	90	70
Phase 6	7/8/2014	1:00 PM	86	92	74
Phase 6	7/9/2014	1:00 PM	88	92	72
Phase 6	7/10/2014	1:00 PM	84	88	82
Phase 7	7/11/2014	3:00 PM	83	86	77
Phase 7	7/12/2014	1:00 PM	84	86	71
Phase 7	7/13/2014	10:00 AM	81	87	78
Phase 7	7/14/2014	1:00 PM	89	90	78
Phase 7	7/15/2014	1:00 PM	84	92	79
Phase 7	7/16/2014	1:00 PM	79	86	76
Phase 7	7/17/2014	1:00 PM	82	83	78
Phase 7	7/18/2014	1:00 PM	82	84	70
Phase 7	7/19/2014	1:00 PM	77	85	70
Phase 7	7/20/2014	10:00 AM	74	81	72
Phase 7	7/21/2014	1:00 PM	80	80	76
Phase 7	7/22/2014	1:00 PM	82	82	77
Phase 7	7/23/2014	1:00 PM	88	89	76
Phase 7	7/24/2014	1:00 PM	84	86	76
Phase 7	7/25/2014	1:00 PM	79	86	70
Phase 7	7/26/2014	9:00 AM	75	80	72
Phase 7	7/27/2014	9:00 AM	78	86	76
Phase 7	7/28/2014	12:00 PM	84	84	78

Table 6. Temperature variations in Fahrenheit of the barn of growing-finishing fed diets supplemented with multi-strain DFM.

Phase	Date	Time	Current Temperature (ºF)	High Temperature (ºF)	Low Temperature (ºF)
Phase 6	6/26/2014	1:00 PM	90	91	84
Phase 6	6/27/2014	1:00 PM	86	90	80
Phase 6	6/28/2014	12:00 PM	80	90	79
Phase 6	6/29/2014	10:00 PM	74	80	72
Phase 6	6/30/2014	2:00 PM	91	91	72
Phase 6	7/1/2014	1:00 PM	75	90	74
Phase 6	7/2/2014	1:00 PM	90	91	81
Phase 6	7/3/2014	2:00 PM	80	92	80
Phase 6	7/4/2014	2:00 PM	92	92	82
Phase 6	7/5/2014	1:00 PM	88	92	82
Phase 6	7/6/2014	11:00 AM	82	92	82
Phase 6	7/7/2014	1:00 PM	88	90	78
Phase 6	7/8/2014	1:00 PM	88	92	78
Phase 6	7/9/2014	1:00 PM	90	92	80
Phase 7	7/10/2014	1:00 PM	86	92	80
Phase 7	7/11/2014	3:00 PM	85	89	80
Phase 7	7/12/2014	1:00 PM	87	88	78
Phase 7	7/13/2014	10:00 AM	84	90	80
Phase 7	7/14/2014	1:00 PM	91	91	82
Phase 7	7/15/2014	1:00 PM	85	91	82
Phase 7	7/16/2014	1:00 PM	81	89	79
Phase 7	7/17/2014	1:00 PM	85	86	75
Phase 7	7/18/2014	1:00 PM	84	84	76
Phase 7	7/19/2014	10:00 AM	76	87	76
Phase 7	7/20/2014	1:00 PM	73	80	70
Phase 7	7/21/2014	1:00 PM	82	83	77
Phase 7	7/22/2014	1:00 PM	84	84	80
Phase 7	7/23/2014	1:00 PM	90	90	81
Phase 7	7/24/2014	1:00 PM	86	90	82
Phase 7	7/25/2014	1:00 PM	80	89	74
Phase 7	7/26/2014	9:00 AM	88	80	71
Phase 7	7/27/2014	9:00 AM	80	90	78
Phase 7	7/28/2014	12:00 PM	86	86	80