THE EFFECT OF MEDIUM CHAIN FATTY ACIDS ON PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

by

Stacie Crowder

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THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. John Scott Radcliffe, Chair

Department of Animal Sciences

Dr. Brian Richert

Department of Animal Sciences

Dr. Darryl Ragland

Department of Veterinary Clinical Sciences

Dr. Roman Pogranichniy

Department of Diagnostic Medicine and Pathobiology,

Veterinary Diagnostic Laboratory,

Kansas State University College of Veterinary Medicine, KS

Approved by:

Dr. Zoltan Machaty

Dedicated to my Family for all their love and support as I chase my dreams. Their belief in me was the fuel to this amazing journey of continued learning to make a difference.
In memory of my Dad, I am who I am because of him. He taught me to take the bull by the horns, always work hard, and reach for the stars.

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is estimated to cost the US swine industry \$664 million in annual production losses. Therefore, the objective of this project was to evaluate the effect of MCFA on PRRSV replication using in vitro and in-vivo studies. The overarching hypothesis was that MCFA would inhibit or reduce viral replication of PRRSV infection in vitro and reduce viral load in-vivo. In the first experiment (Chapter 2), MARC-145 cells were used to determine the effects of individual MCFA (C6, C8, C10, and C12) exposure at concentrations ranging from 1-1000 µg/mL prior to and following inoculation of North American Type II (P-129) or European Type I (Lelystad) PRRSV. Viral replication was determined using FITC labeled IgG anti-PRRSV monoclonal antibody and $TCID_{50}$ was calculated for each concentration. Data were analyzed using the Proc Mixed procedure of SAS. Incubation of MARC-145 cells with caproic acid (C6) at concentrations of 1-1000 µg/mL prior to and after inoculation with Type II North American (P129) or Type I European (Lelystad) PRRSV did not alter viral replication ($P > 0.10$). However, incubation of MARC-145 cells with caprylic (C8), capric (C10), and lauric (C12) acid prior to and after inoculation with Type I and Type II PRRSV did reduce viral replication at concentrations ranging from 100-1000 µg/mL. In general, the effective dose required to reduce ($P < 0.05$) viral replication ($Log_{10}TCID_{50}/mL$) decreased as MCFA chain length increased. In experiment 2 (Chapter 3), the use of MCFA combinations (C8:C10; C8:C12; C10:C12; and C8:C10:C12) to reduce viral replication of PRRSV in MARC-145 cells was investigated. The MCFA combinations were analyzed at six different concentrations ranging from 50-500 µg/mL with North American Type II (P-129) and European Type I (Lelystad) PRRSV. Viral replication was determined as described in experiment 1 (Chapter 2) using FITC labeled IgG anti-PRRSV monoclonal antibody and $Log_{10}TCID_{50}/mL$ was calculated for each concentration.

Data were analyzed using the Proc Mixed procedure of SAS. Incubation of MARC-145 cells with MCFA combinations prior to and after inoculation with Type II North American (P129) and Type I European (Lelystad) PRRSV resulted in reduced viral replication at MCFA concentrations of 200-500 µg/mL and was concentration dependent. Reduction of viral replication with MCFA was further evaluated by independently incubating MARC-145 cells or PRRSV. Results indicated that viral replication was reduced when MARC-145 cells were incubated with MCFA and not when PRRSV was incubated with MCFA. In experiment 3 (Chapter 4), 112 mixed sex pigs (PIC 1050 females x PIC 359 sire), weaned at 21 d of age, weighing 7.5 ± 0.68 kg, were used in a 33d PRRSV challenge study. Pigs were blocked by body weight and sex and randomly assigned to one of four treatments in a 2x2 factorial design with pigs receiving 0 or 0.30% MCFA in the diet and placebo or PRRSV inoculation. Following a 5 d adjustment to diets and rooms, pigs were inoculated with either a placebo (sterile PBS) or Type II North American (P129) PRRSV (1×10^5, $TCID_{50}$/mL) given in 1 mL each intranasal and IM injection. Each room contained 4 pens with 7 pigs per pen and an equal ratio of barrows to gilts within treatment. Diets were formulated to meet or exceed all nutritional requirements (NRC, 2012) and were fed in 4 nursery phases. Feed budgets by phase were 1.13 kg/pig in phase 1, 2.72 kg/pig in phase 2, 6.35 kg/pig in phase 3, and phase 4 fed until the end of the experiment. MCFA (C8:C12) were mixed in a 1:1 ratio (wt:wt), and then mixed with finely ground corn to prepare a premix added to diets at 0.60% to provide 0.30% total MCFA. Control diets used soybean oil mixed with finely ground corn at the same 0.60% inclusion to keep ME levels constant across treatments. Body weights, feed intakes, blood samples, and temperatures were determined or collected on d 0, 3, 7, 10, 14, 21, and 28 post inoculation. Sections of tonsil, lung, and intestines were collected at d 10 post-inoculation from 1 pig per pen and at d 28 from all remaining pigs. Data were analyzed using the PROC Mixed procedure of SAS with

pen as the experimental unit for growth and performance measurements and pig as the experimental unit for viral load analysis. Serum viral load confirmed PRRSV was only detectable in challenged pigs. Body weights were not different ($P > 0.05$) between treatments prior to d 14 post inoculation. Body weights from d 14 to 28 post inoculation were reduced ($P < 0.05$) in PRRSV infected pigs compared to non-infected pigs. Overall ADG and ADFI were reduced ($P < 0.05$) for PRRSV infected pigs compared to non-infected pigs by an average of 18 and 28%, respectively. Body temperatures were not different between treatments. Viral load measured in the lung was not different ($P > 0.05$) between PRRSV infected treatments. Tonsil viral load was not different ($P > 0.10$) between PRRSV treatments. However, there was a trend ($P \leq 0.10$) for an effect of day post inoculation with control-fed, PRRSV-infected pigs having higher viral loads at d 10 post inoculation compared to d 28 post inoculation. Overall, no effects of MCFA on PRRSV viral load or performance were observed during the in-vivo trial. MCFA was effective at reducing viral replication of PRRSV in MARC-145 cells in vitro. However, the results could not be confirmed in the in-vivo experiment. Porcine alveolar macrophages should be used to confirm the in vitro inhibition of PRRSV replication observed in MARC-145 cells. In order to fully understand the application of MCFA to inhibit PRRSV infection in pigs, more studies should be conducted to evaluate the form of MCFA as well as viral inoculation with field strains of PRRSV.

Keywords: MCFA, PRRSV, Viral Replication, Swine

CHAPTER 1.   THE EFFECT OF MEDIUM CHAIN FATTY ACIDS ON PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN PIGS: A LITERATURE REVIEW

1.1   Introduction

The swine industry has been impacted by Porcine Reproductive Respiratory Syndrome Virus (PRRSV) for over 30 years (Brar et al., 2014). First appearance of the virus occurred in the late 1980s in the US and Europe as a mystery swine disease. Virus isolation in 1991 in Europe and 1992 in the US lead to the identification of 2 genotypes: Type I European and Type II North American PRRSV strains. PRRSV has spread rapidly through pork producing countries. PRRSV is an enveloped, positive single strand RNA virus in the Arteriviridae family of viruses in the order of Nidovirales. Transmission of the virus is both vertical, sow to piglet, and horizontal, pig to pig, by nasopharyngeal and aerosol routes.

PRRSV infection results in reproductive failure in sows and gilts as well as respiratory illness in all phases of pig production. Reproductive failure in sows can range from decreased farrowing rates to increased abortions and low viability in liveborn pigs (Butler et al., 2014). Respiratory disease severity increases with the presence of secondary infections leading to high mortality rates in pre-weaned and post-weaned pigs (Amadori and Razzouli, 2014). It has been estimated that PRRSV infection costs the US pork industry $664 million in annual production losses. making it the most economically significant disease facing today's pork producers (Holtkamp et al., 2013). Economic losses from high mortality, decreased growth rates, and respiratory distress in growing pigs account for 55% of production losses. After three decades of PRRSV research, the mechanism for long lasting heterologous immunity is still unclear. Challenges to PRRSV research and subsequent control and prevention includes: rapid viral mutation rate, viral suppression of the

immune response, persistence of infection after known symptoms disappear, and rapid transmission or spread to other animals and locations.

1.2 Porcine Reproductive Respiratory Syndrome Virus

1.2.1 Respiratory Disease and Persistence

Respiratory Disease of PRRSV is characterized by dyspnea, coughing, and sudden death from respiratory distress. Pigs with PRRSV infection often present with rough hair coat, reduced growth rate, dyspnea, and/or coughing (Zimmerman et al., 2006). PRRSV replicates in the lung and lymphoid tissue and certain dendritic cells (Darwich et al., 2010) with the primary site being alveolar macrophages. Macrophages and dendritic cells are essential for activating the adaptive immune response (Murtaugh & Genzow, 2011). PRRSV has been isolated from multiple tissues including tonsils, thymus, lymph nodes, spleen, lung, liver, heart, kidney, and placenta.

Infection of placenta and endometrium are only detected in early and late gestation which leads to placental transfer to the fetus (Karniychuk et al., 2013). PRRSV can replicate in fetal thymus, spleen, and lymph nodes and spread to other fetuses in utero (Novakovic et al., 2016). Inflammatory lesions have been reported in the sow uterus and placenta during PRRSV infection (Lager and Halbur, 1996). PRRSV infection and replication in utero has been linked to loss of placental function, detachment, and degradation which leads to fetal death (Rossow, 1998; Karniychuk et al., 2011).

Viremia and seropositivity can be concurrent for several weeks with persistence up to 157 days (Albina et al., 1998; Otake et al., 2002) after initial infection. Persistence of PRRSV in lymphoid tissue can lead to reinfection as naïve pigs are introduced into the herd and become exposed to circulating strains even if the herd is asymptomatic (Rowland et al., 2003). These

persistent pigs create hot spots within the herd and prevent virus stabilization. Another cause of PRRSV persistence in a herd is infection with a new or mutated virus strain, which effectively starts the disease cycle over again. Management techniques and biosecurity changes have been used by producers in an attempt to break the cycle of infection and stabilize the herd (Mccaw, 2000; S. Dee et al., 2005). Several PPRSV prevention strategies over the years have included reduced animal movements and mixing, heightened biosecurity measures, and herd vaccination. Control or prevention of PRRSV has been challenging due to viral persistence in infected herds, high transmission rates between infected animals, and rapid development of genetic variants. Additional issues develop with genetic evolution of highly virulent strains identified as recently as 2020, increasing the genetic diversity of PRRSV in global pig producing regions (Jiang et al., 2020; Kvisgaard et al., 2020; Park et al., 2020). Genetic diversity in PRRSV has caused a lack of vaccine efficacy and cross protection between strains (Butler et al., 2014).

1.2.2   Genetic Diversity

Understanding the genome of PRRSV is critical to finding effective treatments and prevention of disease. The PRRSV genome is 15 kb in length, single stranded, and positive-sense RNA that encodes nine open reading frames (ORFs). The genome of PRRSV is complex due to the overlapping of genes. Three fourths of the genome is contained in ORF 1a and 1b, and is translated into two polyproteins, replicase proteins pp1a and pp1ab, which are cleaved into 14 nonstructural proteins (nsps; Kimman et al., 2009). The nsps functions are not well known. Their activities include replicase, protease, and polymerase which suggests involvement in genome replication and transcription of subgenomic mRNA. ORF 2a, ORF 2b, and ORF 3-7 code for structural proteins GP2a, GP2b (E), GP3, GP5, GP5, M, and N, respectively (Snijder & Meulenberg, 1998). Structural proteins are required for packaging viral progeny (J Wissink et al.,

2005). Delputte et al. (2002) determined that structural proteins M and GP5 mediate the interaction between PRRSV and the heparan sulfate binding site. Kwon et al. (2008) reported that PRRSV virulence is dependent upon structural and non-structural genes, making PRRSV virulence multigenic. The overlapping nature of the PRRSV genes makes it hard to modify one gene without also affecting the overlapping gene.

PRRSV has a high level of antigenic variation which has been proposed as the reason for differences in virulence and pathogenicity of PRRSV variants. The variation is attributed to mutations during the error-prone replication of RNA. The absence of proof-reading functions in the replication of PRRSV allows for constant generation of variant genomes leading to heterogenous populations of PRRS virus variants (Biebricher & Eigen, 2006). Mutation rates for viral proteins exposed to the PRRSV surface are more variable than internal regions (Chang et al., 2002). The enzymes needed for RNA synthesis, RNA - dependent RNA - polymerase and RNA helicase are encoded in ORF 1b region of the genome. Recombination in PRRSV replication results in the formation of subgenomic mRNA (Murtaugh et al., 2010). Fang & Snijder (2010), reported that RNA synthesis in PRRSV requires genome amplification and production of subgenomic mRNAs for viral structural protein templates. This process is similar to copy-choice recombination with specific RNA signal transcription regulatory sequences (Pasternak, 2001).

1.2.3 Pathogenesis

PRRSV severity varies considerably as a result of virus strain, immune status, genetic susceptibility, and environmental factors. Reproductive morbidities in sows include premature births, late term abortions, stillbirths, mummification of fetuses, weak pigs at birth, decreased farrowing rates, and increased wean to estrus interval (Benfield et al., 1992). The respiratory

morbidities include dyspnea, coughing, and sudden death from respiratory problems. Infected nursery pigs have high mortality rates, poor growth rates, severe bleeding at tail docking, naval bleeding, ataxia, and muscle tremors. Sows may develop fevers and have variable feed intakes. Grow-finish pigs experience fever with decreased feed consumption leading to poor production efficiencies. Secondary viral and bacterial infections are a prime concern with PRRSV infection. Secondary bacterial infections associated with PRRSV include Streptococcus suis, Salmonella cholerae-suis, Glaesserella parasuis, Pasteurella multicida, Mycoplasma hypneumoniae, and Actinobacillus pleuropneumoniae (Li et al., 2017). Secondary viral infections associated with PRRSV infection include Porcine respiratory coronavirus and Influenza A virus. Diagnosis should take all production records and clinical signs into consideration.

PRRSV infects macrophages, compromises the cellular immune response and impairs mucosal function. Gross lesions in respiratory and lymphoid tissue can range from severe to limited. PRRSV can be contained in semen, urine, and feces (Wills et al., 1997). Viral incubation period is 4-5 days with experimental infections. PRRSV impairs pulmonary defense functions through damage to the nasal mucosa, disruption of the bronchial ciliary clearance and destruction of alveolar macrophages (Oleksiewicz and Nielsen, 1999).

1.2.4   Immune Response

Macrophages and dendritic cells are essential for activating adaptive immunity by secreting inflammatory and immunomodulatory cytokines and presenting antigens to T cells (Murtaugh & Genzow, 2011). Infected pigs have a rapid humoral response, and antibodies to PRRSV are detected as early as day 5 post infection (Mateu & Diaz, 2008). These early IgM and IgG responses do not provide neutralizing antibody protection against the virus (Yoon et al., 1994; Xiao et al., 2010). Initial antibodies can cause an antibody-dependent enhancement by promoting virus entry

into target cells (Yoon et al., 1996). In cell culture PRRSV can interfere with interferon signaling through nonstructural proteins in a strain specific response (Murtaugh & Genzow, 2011). Mateu & Diaz, (2008) reported that neutralizing antibodies are not detected in the first 4 weeks post infection.

PRRSV modulates the host's immune response. Xiao et al., (2010) reported that PRRSV induced an inflammatory response during early infection, resulting in apoptosis of infected cells and reduced immune cells, thereby leading to an immunosuppressive response. Macrophages do not kill the virus; the virus kills macrophages by rapidly producing more virus. It is estimated that up to 40% of alveolar macrophages are destroyed by PRRSV (De Baere et al., 2012). The reduction in alveolar macrophages reduces the immune response in the lungs of pigs infected with PRRSV. Reductions in lung immune cells increase the incidence of secondary infections associated with porcine respiratory disease complex (Brockmeier et al., 2014). Since porcine alveolar macrophages are the target cell for PRRSV, several studies have been conducted to characterize the interaction between alveolar macrophages and PRRSV. It has been reported that PRRSV inhibits type I interferons (IFN; Albina et al., 1998) and induces interleukin-10 (IL-10; Suradhat & Thanawongnuwech, 2003). Genome wide transcription of alveolar macrophages post- PRRSV infection results in a strong upregulation of beta interferon 1 expression and a weak upregulation of IL-10 and tumor necrosis factor α (TNF-α; Genini et al., 2008). Miller et al. (2008) identified over 400 unique alterations in porcine alveolar macrophage gene expression after PRRSV infection.

PRRSV has been reported to inhibit IFNα responses (Mateu & Diaz, 2008). It has also been suggested that PRRSV isolates of the same strain can differ in their ability to affect IFNα (C.-H. Lee et al., 2004). Kimman et al. (2009), reported that IFNα inhibited PRRSV replication.

Inhibition of IFNα would also impair development of an adequate T helper type 1 immune response (Mateu & Diaz, 2008). PRRSV alters cytokine production in macrophages and dendritic cells, and modifies antigen presenting molecules (Wang et al., 2007). It has been reported that PRRSV reduces expression of major histocompatibility complex-I in dendritic cells (Loving et al., 2007). Research with infectious and inactivated PRRSV has resulted in down regulation of the expression of major histocompatibility complex I and II along with CD14 (Wang et al., 2007). After infection with PRRSV, specific IFNγ secreting cells are not detected until 21 days post-infection (Díaz et al., 2005, 2006). IFNγ secreting cells are CD4$^+$ CD8$^+$ with a small amount coming from CD4$^-$/CD8αβ$^+$ T cells (Meier et al., 2003). Darwich et al. (2010) reported that IFNγ is used as a measurement of cell mediated immunity to PRRSV. IL-10 mRNA is increased in bronchoalveolar lavage fluid in pigs infected with PRRS virus (Thanawongnuwech et al., 2004). Induction of immunosuppressive cytokines IL-10, IL-4 and transforming growth factor β (TGFβ) are involved in immune evasion and secondary infections with PRRSV infection (Murtaugh & Genzow, 2011; Chen et al., 2019). However, there are still gaps in understanding the full mechanism of immune evasion.

In general, neutralizing antibodies are critical to control and clear viral infections but are delayed with PRRSV infection. Several studies have reported that PRRSV viremia has been resolved before detection of neutralizing antibodies (Nelson et al., 1994; Díaz et al., 2006). Neutralizing antibodies are not detected until 4 weeks post-infection and are mainly targeting GP5, the major neutralization epitope B (Nelson et al., 1994). There is also a decoy epitope on the GP5 N-terminal ectodomain identified as epitope A (Ostrowski et al., 2002). The decoy epitope could alter the immune response to epitope B causing a delay in neutralizing antibodies. Viremia may be influenced by PRRSV susceptible macrophages (Díaz et al., 2006). If susceptible macrophages

are reduced or exhausted by apoptosis, then infection will spread in macrophage rich organs (Mateu & Diaz, 2008). Neutralizing antibodies have been reported to block PRRSV infection of macrophages in vitro, through a reduction in attachment and internalization (Delputte et al., 2004). Mateu & Diaz, (2008) reported that transfer of neutralizing antibody titers of 1/8 to 1/16 protected piglets and sows from viremia and reproductive failure. However, titers of 1/32 are needed for sterilizing immunity, suggesting that vaccine development should be targeting a neutralizing antibody titer of 1/32.

Toll like receptors (TLR) are important in the host antiviral response. There are conflicting reports on the impact of PRRSV on TLR 3 expression. Chaung et al., (2010) reported an inhibition of TLR3 and TLR7 with PRRSV infected porcine alveolar macrophages and immature dendritic cells in vitro. However, in vivo /ex vivo PRRSV infection studies reported the opposite with an increase in TLR 3 as well as TLR 2, TLR 4, TLR 7, and TLR 8 (Sang et al., 2008; Liu et al., 2009; Miguel et al., 2010). Darwich et al. (2010) reported that TLR 3 is important in viral replication. Miller et al., (2009) reported activated TLR 3 signaling decreased viral replication. These variable results suggest there could be strain differences and interference in TLR 3 signaling (Loving et al., 2007; Darwich et al., 2010;).

There are three cellular receptors/mediators known in the pathway of PRRSV entry into macrophages (De Baere et al., 2012): 1) heparan sulphate is an important attachment factor on macrophage surfaces, 2) porcine sialoadhesin (PoSn) influences binding and internalization of virus with the receptor, and 3) hemoglobin scavenger receptor CD 163 is involved in uncoating and release of the viral genome (Van Breedam et al., 2010). CD 163 has been considered to have a critical role in PRRSV infection. CD 163 is part of the group B scavenger receptor cysteine rich (SRCR) protein family. It is an extracellular protein containing nine SRCR repeats, has an

intracellular cytoplasmic tail, and a transmembrane section (Welch & Calvert, 2010). CD 163 expression has been correlated with the immune response to infection CD 163 is low in immature monocytes and increases with maturation of undifferentiated monocytes (Sánchez et al., 1999). Following an inflammatory stimulus, Pérez et al., (2008) was able to detect soluble CD 163 in porcine alveolar macrophages in culture.

Multiple variants of CD 163 have been identified with differing tail lengths (Welch & Calvert, 2010). The cytoplasmic tail may have an inhibitory effect on virus replication since viral production was reported higher in cells with tailless CD163 receptors (Lee & Lee, 2010). Several studies have examined the domains of CD163 with various deletion mutants. The SRCR 5 domain of CD 163 has been identified as critical for PRRSV infection (Welch & Calvert, 2010). Xiao et al. (2010) reported that the concentration of CD 163 on the surface of macrophages influences viral replication and pathogenicity of PRRSV. This has been demonstrated with non-permissive cells that have been transfected with porcine CD 163 and become permissive cells resulting in replication of PRRSV (Lee et al., 2010). Patton et al., (2009) incubated CD 14$^+$ monocytes with IL-10 and observed an increase in CD 163 expression with increasing PRRSV infection. CD 163 increases after PRRSV infection, increasing the amount of PRRSV inside macrophages, which has been suggested to increase IL-10 expression, in turn increasing CD 163 expression, resulting in increased PRRSV susceptibility (Xiao et al., 2010).

1.3   Swine Immune System

The immune system is a complex system that that can be broken down into three different phases that include barriers, innate, and adaptive immune processes. Barriers are divided into physical, chemical, and microbiological. Physical barriers include skin, epithelial cell tight junctions, peristalsis in the intestine, mucocilliary movement of the respiratory tract, tears, and

nasal cilia (Okumura and Takeda, 2016). Chemical barriers consist of fatty acids, antimicrobial peptides, pH, and antimicrobial enzymes. Microbiological barriers involve commensal bacterial of the skin, gastrointestinal tract, and mucosal surfaces. The role of these barriers is to prevent pathogens from entering the body and causing infection.

The innate immune response is immediate, usually within hours to days and consists of cells and soluble factors. Cells of the innate immune system include granulocytes, macrophages, mast cells, NK cells, and dendritic cells (Mair et al., 2014). Soluble factors include complement proteins, acute phase proteins, interferons, and other cytokines (Shishido et al., 2012). Activation of the innate immune system is initiated with pathogen recognition and tissue damage leading to recruitment of leukocytes and inflammation. Innate immune system activation does not result in memory induction and therefore dendritic cells are a critical link to the adaptive immune system as they present the pathogen- derived antigen (Mair et al., 2014).

The adaptive immune system is delayed, usually taking days to weeks to develop. Cells and tissues of the adaptive immune system consist of B and T lymphocytes, and primary (thymus and bone marrow) and secondary (ileal Peyer's patch, lymph nodes, spleen, and mucosal lymphoid tissues) lymphoid tissues,. Activation of the adaptive immune system results in memory B and T cells providing a stronger response to a second exposure of the same antigen that induced the initial response. The response of B and T lymphocytes is specific to the antigen receptors on the B and T lymphocytes (Rahe et al., 2017).

1.3.1   Innate Immune System

Innate immune system cells originate in bone marrow and consist of myeloid or lymphoid cells. Myeloid cells are granulocytes, monocytes and macrophages, dendritic cells, and mast cells. NK cells are innate lymphoid cells (Shishido et al., 2012). Granulocytes consist of three cell types:

neutrophils, eosinophils, and basophils. Neutrophils are the most abundant and present first at the site of infection or tissue damage (Kumar & Sharma, 2010). Neutrophils kill bacteria or other microbes through multiple mechanisms including the production of enzymes, defensins, reactive oxygen radicals. nitric oxide, and neutrophil extracellular traps (Brinkmann, 2004). Circulating neutrophils have a short life span usually lasting 48-72 hours. Eosinophils contain various enzymes and basic proteins that are toxic to parasites and cells (Magyar et al., 1995). Eosinophils secrete cytokines and lipid molecule leukotrienes that are proinflammatory (Masure et al., 2013). Circulating eosinophils role is primarily in response to allergic reactions, and their life span is usually days to weeks. The third type of granulocyte is basophils, and they are also involved in allergic reactions and release inflammatory mediators (Uston et al., 2007). Basophils usually have a life span of days. Granulocytes have roles in response to acute inflammation, parasitic infections, and allergic reactions (Mair et al., 2014).

Other myeloid cells include monocytes and macrophages. Monocytes circulate in the blood and differentiate into macrophages in tissues (Bogunovic et al., 2009; Guilliams et al., 2013). The functions of macrophages include phagocytosis, killing of pathogens, secretion of inflammatory mediator cytokines and nitric oxide, and tissue repair (Geissmann et al., 2010). Roles of monocytes and macrophages include immune surveillance, antimicrobial response, and antigen presentation (Hume, 2008). Dendritic cells are closely related to macrophages, are derived from monocytes, and have a role in immune surveillance and antigen presentation (Merad et al., 2013). Dendritic cells are present in lymphoid and non-lymphoid tissues. Mast cells reside in connective tissue and mucosal lamina propria and have a role in immune surveillance and initiation and amplification of inflammatory responses through secretion of histamine, cytokines, prostaglandins, and leukotrienes. The lymphoid cells of the innate immune system are NK cells.

NK cells are involved in the immune response to tumor cells and viral infections. They function by killing infected or neoplastic cells and secreting cytokines (Denyer et al., 2006). NK cells do not express antigen specific receptors.

The innate immune systems' role is to detect pathogens and tissue damage by pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) recognized through pattern recognition receptors (PRRs; Kumar et al., 2011). PRRs are localized in cell membranes, endosomes, and cytoplasm and lead to secretion of cytokines, chemokines, and antimicrobials to kill infected or altered cells (Szabo & Rajnavolgyi, 2013). Bacteria, viruses, and fungi have specific PAMPs that are not part of mammalian cells which allows PRRs to alert the innate immune system (Kumar et al., 2011). DAMPs alert the innate immune system to cellular damage by detecting ATP or uric acid in the extracellular environment. Many of the innate immune cells secrete cytokines to communicate within the immune cells or to non-immune cells. There are multiple classifications of cytokines including interleukins, interferons, tumor necrosis factor, growth factors, and chemokines (Haddad et al., 2002). Through communication they can invoke autocrine, paracrine, or endocrine responses.

1.3.2   Adaptive Immune System

The adaptive immune system's B and T lymphocytes are developed in primary lymphoid organs in an antigen independent process or in secondary lymphoid organs in an antigen dependent process (Sinkora & Sinkorova, 2014). Circulating B and T lymphocytes express specific antigen receptors, are naïve lymphocytes until exposure to their specific antigen, and are involved in immune surveillance. Once a naïve lymphocyte is exposed to its specific antigen it becomes a lymphoblast. Lymphoblasts proliferate and B cells differentiate into memory cells or plasma cells to produce immunoglobulins, and T cells differentiate into memory cells or effector T cells (Slifka

et al., 1998). Lymphocytes and sub-populations of lymphocytes are identified by their specific membrane molecules. Cluster of differentiation (CD) classifications of membrane molecules allow for identification between B and T cells. CD3 is present on all T cells and subpopulations of T cells are identified as T helper cells with CD4 and cytotoxic T cells with CD8. B cells are identified by the presence of CD21 membrane molecules (Šinkora et al., 1998).

Primary lymphoid organs are the site of development for naïve lymphocytes. Bone marrow contains hemopoietic stem cells that produce lymphoid precursor cells to B and T lymphocytes and is the site of B cell development in some mammals. Swine, ruminant, and canine B cell development occurs in ileal Peyer's patches. Avian B cell development occurs in the bursa fabricius. T cell development occurs in the thymus, which is a primary lymphoid organ. Primary lymphoid tissues undergo involution or regression when the animal reaches sexual maturity.

Secondary lymphoid organs are involved in immune surveillance by naïve lymphocytes. Interaction between antigens and naïve lymphocytes leads to activation of the adaptive immune response. Tissues with antigen presenting cells interact with T cells which in turn communicate with B cells to initiate production of memory plasma cells and include lymph nodes, spleen, and mucosal lymphoid tissues. Lymph nodes are encapsulated organs that contain a cortex, paracortex, and medulla. Afferent lymphatics deliver antigens to lymph nodes, and naïve lymphocytes enter the lymph node by high-endothelial venules in the paracortex and exit via the efferent lymphatics (Binns & Pabst, 1994). Swine have inverted lymph nodes with the cortex in the center surrounded by the paracortex and minimal medulla (Rothkötter, 2009). Lymphocytes leave the swine lymph node via the bloodstream. The cortex contains primary and secondary follicles. Primary follicles are made up primarily of naïve B cells with secondary follicles forming after antigen stimulation with a germinal center of activated B cells. The paracortex of the lymph node contains T cells and

dendritic cells. Plasma cells and macrophages are contained in the medulla of the lymph node. Lymph nodes are positioned throughout the body and used for surveillance within their respective regions. Mesenteric lymph nodes are an important part of the adaptive immune response in the small intestines and bronchial lymph nodes in the lungs.

Spleen white pulp is a secondary lymphoid organ that consists of follicles and periarterial lymphoid sheaths. The white pulp is separated from the red pulp by the marginal zone. The spleen filters blood through the marginal zone where macrophages trap and transport antigens into the white pulp (Rothkötter, 2009). Other secondary lymphoid tissues consist of mucosal-associated lymphoid tissue (MALT). There are multiple classes of MALTs in the gut, conjunctiva, bronchus, and nasal associated lymphoid tissues (Gebert & Pabst, 1999). MALT inductive sites are where the adaptive immune response is initiated or induced and include Peyer's patches, tonsils, bronchus-associate lymphoid tissue (BALT), and gut-associated lymphoid tissue (GALT). Diffuse sites of MALT are located throughout the lamina propria and epithelium of mucosal tissues (Cesta, 2006). Adaptive immune surveillance is carried out by circulating lymphocytes that migrate through the secondary lymphoid tissues connected by blood or lymph vessels. Activated lymphocytes change their migration and move to non-lymphoid tissue where the antigen or site of infection is located (Schulz et al., 2009).

1.3.3   Immune Response to Viruses

Viruses are classified broadly by their genetic material as either DNA or RNA viruses. Viruses are obligate intracellular pathogens. The viral nucleic acid core is surrounded by a protein capsid with some large viruses having a lipid envelope encompassing the capsid. Viral infection is dependent on specific receptors on host cells. Viruses bind to these specific cellular receptors

to enter the cell, replicate, and release new viral copies. Viral infection can lead to multiple effects in host cells ranging from host cell death to neoplastic transformation to latency.

Innate immune response to viruses starts with viral DNA or RNA detection by TLRs and RLRs in the cytoplasm or endosome (Pang & Iwasaki, 2011). Type 1 IFN induces antiviral proteins via autocrine or paracrine mechanisms that interfere with viral replication, activate NK cells, and increase antigen presenting cells (Komatsu et al., 1996). Virus infected cells are detected by effector cells and soluble molecules (Rahe et al., 2017), leading to activated innate immune cells inducing the release of chemokines to recruit more effector cells, causing inflammation and viral elimination. The adaptive immune response is activated by dendritic cells via transfer of the viral antigen into the lymph system.

The adaptive immune system responds with activated B and T lymphocytes that have specific viral receptors to the virus that is present and will proliferate and become effector cells (Rahe et al., 2017). The effector cell antibodies or T cells migrate to the site of infection leading to virus elimination. Chronic infection or host cell death occurs if the virus is not eliminated. Effector antibodies function by blocking binding to cellular receptors through neutralizing antibodies, complement mediated lysis opsonization, or antigen dependent cell mediated cytotoxicity. T cell response to viral infection involves Th1 cell mediated immunity

There are viruses that have developed methods to evade the immune response and persist in the host. Viruses can bind to the Fcγ-receptor on macrophages and facilitate uptake and infection (Sips et al., 2016; Nikitina et al., 2018). Viruses can secrete proteins that inhibit or mimic cytokine activities. For example Pox viruses secrete IL-10 like protein or IL-1 binding protein (Spriggs et al., 1992). Viruses can alter MHC1 antigen presentation, decrease detection of a viral infection through interference of MHC1 transport to the cellular surface, decrease expression of TAP1 and

TAP2, or through MHC1 degradation (Ambagala et al., 2005). Another way a virus can evade the immune system response is via antigenic variation or mutation, which prevents the binding of antibodies (Drake and Holland, 1999). This is common with RNA viruses.

PRRSV immune modulations have been studied for over 30 years to detail its evasion or suppression of the immune system (Rahe et al., 2017). PRRSV infects macrophages that are involved in regulation of the immune response. Type I IFN are increased with viral recognition by cellular cytoplasmic and endosomal receptors (Lawson et al., 1997). PRRSV inhibits the expression of type 1 IFN allowing the virus to replicate undetected (Nazki et al., 2020). The initial humoral response to PRRSV infection lacks neutralizing immunity as neutralizing antibodies are not detectable until 4 weeks post infection. The delay in detection of neutralizing antibodies signals suppression of the adaptive immune response. Inhibition of IFNα modulates the antigen presenting cells, which in turn decreases activation of T and B lymphocytes interfering with the Th1 and Th2 immune response (S.-M. Lee et al., 2004; Sun et al., 2012; Wang et al., 2014). The detection of neutralizing antibodies after 4 weeks post-infection demonstrates that the pig's immune system does detect the PRRSV. Decoy epitopes of PRRSV may interfere with recognition and binding to the neutralizing epitope (Thaa et al., 2013). Decoy epitopes are N-glycan membrane proteins found on the PRRSV. There are PRRSV strain differences that exist in how many and the structure of the N-glycan that flank neutralizing epitopes, which could explain the difference in immune response between PRRSV strains and why cross protection is not achieved with heterologous strains (Ansari et al., 2006).

1.4   Medium Chain Fatty Acids

1.4.1   Types and Form of Medium Chain Fatty Acids

Fatty acids naturally occur in fats and oils and can be saturated or unsaturated. Saturated fatty acids are less prone to oxidation and rancidity which is an issue with fats used in animal feed. Fatty acids can be used as an energy source, cellular membrane components, or lipid mediators (Calder, 2008). Medium chain fatty acids (MCFA) are saturated fatty acids with a carbon chain length ranging from 6-12 carbons and include caproic acid (C6), caprylic acid (C8), capric acid (C10), and lauric acid (C12). Their carbon chains are attached to a carboxyl group that is hydrophilic and the carbon chain is lipophilic giving MCFA a similar hydrophilic/lipophilic balance as that of bacterial cell membranes (Park et al., 2018). Fat sources of MCFA are palm kernel oil, coconut oil, and milk from some animals (Zentek et al., 2011). MCFA are found in different forms. The largest structure is a glyceride where the MCFA is bound to the glycerol backbone found in triglyceride, diglyceride, or monoglyceride structural configuration. The MCFA that is bound to glycerol will need to be cleaved by lipase in order to be active in the digestive tract. MCFA used in animal feeds can also be in the salt form bound to a calcium or sodium molecule. The salt form is dissociated in the acidic environment of the stomach releasing the free MCFA. Free MCFA can be used in animal feed and do not require lipase activity or an acidic environment to be functional. The form of the MCFA influences the function as well as the inclusion needed in feed to see a response (Ravindran et al., 2016). MCFA used in animal feed formulations as triglycerides are fed at higher rates to provide free fatty acids when compared to free fatty acids in diet formulations. Previous research has reported the benefit of dietary supplementation of MCFA in feed biosecurity and growth performance in swine (Cochrane et al., 2016; Lerner et al., 2020).

1.4.2 Medium Chain Fatty Acid Digestion and Metabolism

Fat digestion breaks down the triglyceride molecules into diglycerides, monoglycerides and free fatty acids that are packaged into micelles and travel via the lymphatic system to the liver for further metabolism. Emulsification of triglycerides is initiated in the stomach by lipases from gastric and salivary origin and account for less than 30% of total hydrolysis with most of the triglyceride digestion carried out by hydrolysis via pancreatic lipase in the small intestines. Transportation of lipid fractions into intestinal enterocytes is facilitated by the formation of micelles through mixing with bile salts. Type of dietary fat is a primary determinant of digestion, metabolism and absorption and is influenced by the degree of saturation and carbon chain length (Ravindran et al., 2016). MCFA, due to their short carbon chain lengths, can bypass micelle formation and be absorbed through by the intestinal enterocyte and used directly as an energy source or transported via the portal vein to the liver where they are oxidized more easily than longer chain fatty acids and in that respect act more like glucose than fats. MCFA do not require the aid of binding proteins, as with longer chain fatty acids, can cross the mitochondrial membrane, and are more readily oxidized by intramitochondrial medium chain acyl CoA synthetases (Noguchi et al., 2002). Free fatty acids interact with immune and nonimmune cells through G protein coupled receptors (GPCR) along the intestine (Wang et al., 2006).

A specific GPCR, GPR84, is activated by MCFA and has high affinity for capric (C10) and lauric (C12) acid. Research has shown that GPR84 is highly conserved in vertebrates (Huang et al., 2014) and is expressed in various tissues including spleen, lung, thymus, liver, colon, and immune cells including granulocytes, monocytes, macrophages, and B and T cells. Activation of GPR84 in the presence of MCFA increases the inflammatory response by increasing IL-12p40 production, which is a subunit of IL-12 (Huang et al., 2014). IL-12p40 is expressed in monocytes and macrophages following stimulation with PRRSV as early as 1-day post infection. Kobayashi

et al. (1989) reported that IL-12 serves as an important link between the innate and adaptive immune responses. Increased expression of GPR84 has been reported under inflammatory conditions in multiple studies (Suzuki et al., 2013; Huang et al., 2014). Tsuzuki et al., (2006) reported that the chemotaxic response to activated GPR84 was specific to neutrophils. Fatty acids C6, C8, and C10 have been shown to polarize naïve T cells to a Th17 and Th1 phenotype (Haghikia et al., 2015). GPR40, another GCPR, has also been reported to be activated by MCFA. This is important because GPR40 is highly expressed in monocytes, indicating that MCFA have the potential to influence immune modulation (Briscoe et al., 2003).

1.4.3 Medium Chain Fatty Acids Antimicrobial Properties.

MCFA have been reported to have antimicrobial properties when tested against algae, fungi, bacteria, and viruses (Desbois & Smith, 2010; Fischer et al., 2011) MCFA have been researched as alternatives to antibiotics in pig production (Decuypere & Dierick, 2003). The use of antibiotics in US animal production systems is now under increased regulatory oversight with mandatory veterinary feed directives (VFD) and there is additional pressure to reduce the overall antibiotic footprint of animal agriculture. Improvements to animal performance have been reported when MCFA are added to feed (Thomas et al., 2020). Research on intestinal morphology and gut microbiota have reported increased villi heights and crypt depths as well as modified microbiota with the use of MCFA in pig diets (Dierick et al., 2003; Calder, 2008; Zentek et al., 2012). The mode of antimicrobial action of MCFA appears to be multifaceted. The hydrophilic/lipophilic balance of MCFA is similar to that of the cellular membrane of bacteria, giving MCFA the ability to attach and penetrate the cellular membrane. Degradation of the microbial cell membrane is facilitated by MCFA penetrating the membrane and causing breakage or pores that allow cellular contents to escape (Yoon et al., 2015 and 2018). Another mode of action of MCFA is that they

enter the cytoplasm of the bacterial cell and dissociate, lowering the internal pH of the cell, causing cell death (Rossi et al., 2009). MCFA have also been reported to reduce the replication of bacteria by interfering with DNA replication by intercalating into bacterial DNA (Herman & De, 2017). Free fatty acids can interfere with the electron transport chain, oxidative phosphorylation, and membrane enzymes. These actions of free fatty acids disrupt bacterial energy production and nutrient uptake, thereby inhibiting bacterial growth (Desbois & Smith, 2010; Yoon et al., 2018). MCFA have been reported to inhibit bacteria that are common challenges to animal production systems including *escherichia. coli, salmonella, clostridium, and campylobacter*. The rise in antimicrobial resistant bacteria have become an issue in animal production. The mode of action for MCFA make them an increasingly interesting alternative as it is difficult for bacteria to mutate leading to resistance (Yoon et al., 2018).

The antibacterial effects of MCFA have made them the subject of much research investigating alternatives to antibiotics, and more recently feed mitigation. Research evaluating the survivability of pathogens in feed, reported that bacteria and viruses can survive in feed and feed ingredients (Dee et al., 2014; Cochrane et al., 2016; Dee et al., 2020; Lerner et al., 2020). Pet food manufacturers have been under increased regulatory standards since the 2011 Food Safety Modernization Act from the US Food and Drug Administration which states a zero-tolerance policy regarding salmonella contamination in pet foods. The main issue with dry pet food is post manufacturing contamination. The extrusion process used to manufacture dry pet food utilizes high temperatures which should eliminate bacteria. Therefore, the focus has been on ingredients added or sprayed on after processing. Additives, enzymes, flavorings, fats, and oils are applied post-application to dry pet food and can result in recontamination after processing. MCFA have

been reported to reduce salmonella (Cochrane et al., 2016; Dhakal & Aldrich, 2020) in raw materials and dry pet food.

Swine feed has come under heavy scrutiny in the last few years following Porcine Epidemic Diarrhea Virus (PEDV) outbreaks in 2013-2014. Feed has been tested as the vector for disease transmission and it has been determined that feed infected with PEDV can result in viral transmission to pigs (Dee et al., 2014). Ingredient sourcing and the potential for contamination coming through global trade has raised alarms about the introduction of a foreign animal disease. Transboundary model testing has been conducted to determine viral survival during shipment using similar transport times and conditions as global trade routes (Dee et al., 2016; Dee et al., 2018). Treating feed with MCFA has been reported to prevent PEDV infection in a bioassay (Cochrane et al., 2016;). Further studies have been conducted to confirm that MCFA can reduce viral transmission of PEDV (Dee et al., 2020; Lerner et al., 2020). The hypothesis of how MCFA reduce viruses in feed has focused on the interaction of MCFA with the viral envelope. The viral envelope is a bilipid membrane and MCFA have been reported to disrupt the integrity of the envelope leading to reduced pathogenicity of the virus (Thormar et al., 1987).

Research results reporting the benefits of using MCFA as a feed mitigation strategy against PEDV have led to evaluating their use with other envelope viruses including PRRSV and African Swine Fever Virus (ASFV). Treating feed ingredients with 1% MCFA (C6:C8:C10) before or after viral inoculation with ASFV resulted in no functional virus using an in in vitro and in vivo pig bioassay (Niederwerder et al., 2020). Treating complete swine feed with MCFA has also been reported to reduce the disease transmission of PEDV and PRRSV through feed, as demonstrated utilizing an ice block method of inoculating feed in the feed bin to simulate using feed as the vector for viral transmission. MCFA treated feed when tested in this model resulted in reduced disease

transmission with PEDV and PRRSV (Dee et al., 2020). PRRSV is not typically thought of as a feed transmissible virus. PRRSV enters the nasal passages via aerosol transmission and infects alveolar macrophages, leading to respiratory distress. . Further research focused on determining the effects of MCFA on PRRSV directly, as well as determining if MCFA fed to pigs could reduce viral load or persistence of PRRSV infection are critical to understanding the practical applications of MCFA. The nature of swine production and large group housing presents a challenge to disease management. Many times, it is not a single pathogen issue but instead multiple pathogens and in the case of PRRSV, secondary infections that cause production losses and mortality. The multiple modes of action associated with MCFA coupled with the immune modulation of free fatty acids leads to the hypothesis that MCFA could support improved immune function and animal health.

1.5   Swine Nutrition and Health

1.5.1   Nutritional Impact on Health

It has been generally recognized that nutrition can impact health in animals and humans. The swine industry has researched nutrient requirements and the impact of deficiencies on performance and health. However, as the industry continues to evolve, a better understanding the focus is shifting from requirements that maximize growth to a more complex understanding of nutrient requirements needed to optimize growth, immune responsiveness and animal health. Nursery scours have been an issue for swine and pharmacological levels of zinc and copper have been used to firm up stools and improve performance (Hill et al., 2000; Højberg et al., 2005; Hollis et al., 2005; Shelton et al., 2011; Walk et al., 2015). Enzymes are also added to swine diets to improve performance and environmental footprint, including phytase, xylanase, and carbohydrase. When linking nutrition to health, a focus should be placed on gut bacterial populations and the

impact of dietary nutrients on intestinal function. Nursey pigs are transitioned from milk-based diets to grain-based diets at weaning and it has been well established that intestinal epithelial lining is altered post-weaning (Nabuurs et al., 1993; Pluske et al., 1997; Pié et al., 2004; Gilbert et al., 2018). These intestinal changes that occur at weaning coupled with low feed intake immediately post-weaning lead to reduced digestion and increases in undigested protein reaching the lower gut (Salgado et al., 2002; Heo et al., 2015). In particular, *E. coli* and *clostridia* are proteolytic bacteria and proliferate on undigested protein. Therefore, the use of enzymes in nursery diets could impact health by improving the digestion of nutrients and reducing the proliferation of pathogenic bacteria in the gut microbiota. Multiple feed additives have been reported to improve growth performance through improved digestion (direct fed microbials, enzymes, MCFAs, organic acids, and phytogenics; Zentek et al., 2011; Windisch et al., 2008; Zeng et al., 2015; Zimmermann et al., 2016).

Nutritional impacts on health require the intercommunication between gut tissue, the central nervous system, and the immune system (Rehfeld, 2004; Janssen & Depoortere, 2013). The effects regulated through the gut enteroendocrine cells include modification of intake, gastric passage time, stimulation of digestive enzymes, and immune responses (Furness et al., 1999; Ahlman & Nilsson, 2001;). The intestinal lining has cells that monitor luminal contents to detect nutrients and support digestive processes. Free fatty acids, when detected in the intestinal lumen, induce the release of bile salts and pancreatic lipases via activation of GPR40 (Furness et al., 2013). This causes the release of cholecystokinin, which in turn stimulates secretion of digestive enzymes (amylases and proteases) and signals the vagal efferent pathway, linking dietary fatty acids to improved nutrient digestion (Furness et al., 1999). Phytogenic additives act on receptors in the intestines called transient receptor potential channels (TRP) that impact taste and smell (Ishimaru

& Matsunami, 2009; Holzer, 2011). TRP receptors have been found in gastrointestinal mucosa of mice (Furness et al., 2013) providing evidence of endocrine signaling and sensory function of intestinal cells. Understanding the vast interaction of the gut with other regulatory systems is still not complete but does provide new targets for impacting health and performance in the animal as well as new targets for therapeutic products to prevent infection and disease spread.

1.5.2   Intestinal Function and Integrity

Intestinal barrier function is the main defense mechanism to protect the animal from microorganisms and pathogens in the diet and environment (Assimakopoulos & Vagianos, 2007; Camilleri, 2019). The layers of the intestinal barrier consist of a biological barrier consisting of the gut microbiota, an immune barrier made up of MALT and GALT, and a mechanical barrier consisting of the intestinal epithelial and endothelial cell lining (Assimakopoulos, 2011; Wells et al., 2017). Specialized epithelial cells secrete mucins to create mucus that is a thick gel like layer as a first line of defense (Johansson et al., 2008; Kamada & Núñez, 2014). Mucus contains antimicrobial peptides such as defensins and secretory IgA to bind bacteria and help maintain barrier function (Turner, 2009). Particles and substances are transported across the barrier via transcellular and paracellular routes (Wells et al., 2017). Transcellular transport involves movement through intestinal epithelial cells and requires selective transporters for example sodium coupled transport for glucose (Camilleri et al., 2019). Paracellular transport is via the space between intestinal epithelial cells and is regulated by tight junctions and adheren junctions (Turner, 2009). There is an interdependence of both transcellular and paracellular transport to maintain a functioning gut barrier. Active transcellular transport requires a functioning tight junction and activates an intracellular signaling to influence passive transport of ions and water through the paracellular tight junctions (Camilleri et al., 2019). Dietary nutrients influence intestinal barrier

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function by interaction with GPCR that induces the regulation of metabolic functions of digestion, immune response and cellular signaling (Wu et al., 2011; Sun et al., 2017; Tan et al., 2017). Maintaining a functioning intestinal barrier requires complementary and cross functional regulation of gut microbiota, mucus production, epithelial cell lining, and the mucosal immune system.

1.5.3   Interaction of the Gut and the Immune System

The gut contains 70-80% of the animal's immune cells (Castro & Arntzen, 1993) providing a link between the gut and immune system. This link establishes the foundation to understanding the impact of nutrition and nutritional components on animal health. Intestinal epithelial cells interact with gut immune cells via presenting antigens to dendritic cells and production of cytokines, chemokines, hormones and enzymes (Vitale et al., 2016). MCFA provided through feed can have direct and indirect effects on animal health. The direct effect of MCFA is to reduce pathogens in the gut and serve as an energy source to the epithelial cells. Research on MCFA metabolism has reported that up to 49% of C10 and 72% of C12 reaching the duodenum is recovered in the portal blood in rats (McDonald et al., 1980; Papamandjaris, 1998).

When pathogens are reduced in the gut, it has an energy sparing effect by redistributing energy that would have been used on an immune response towards growth and performance. Indirect effects of MCFA result from changes to gut microbial populations that impact immune function (Yoon et al., 2015). Several research studies have reported the effect of diet on gut microbial populations (Zhou et al., 2016; Liu et al., 2018; Yu et al., 2019; Zhao et al., 2020). Studies have reported a link between gut microbes and distal organ immune functions such as Gut-Lung, Gut-Brain, Gut-Liver providing evidence for the role it plays in infectious and chronic

diseases as well as immune responses (Xiao et al., 2011; Furness et al., 2013; Budden et al., 2017; Ge et al., 2021). Suggested modes of action for gut mediated immune functions to pathogens involve PRRs such as TLR and microbial-associated molecular patterns (Chen et al., 2017). Gut microbial immunity is not fully understood but has the potential to increase the application of nutritional strategies to support animal health via microbial derived immunoglobulins (Abaidullah et al., 2019).

In summary, understanding that PRRSV infection leads to increased inflammation and is immune suppressive provides the basis for providing MCFA through the diet to support immune function. MCFA are immune modulatory and impact T cell signaling through GPR84 (Huang et al., 2014) and monocyte differentiation through GPR40 (Briscoe et al., 2003). MCFA C6, C8, C10, C12 have been reported to polarize naïve T cells to a Th17 and Th1 phenotype (Huang et al., 2014; Haghikia et al., 2015). MCFA influence neutrophils through the activation of GPR84. The research presented earlier on feed mitigation using MCFA demonstrates that MCFA can inhibit viral infection in in vivo studies. These findings suggest that it is possible to influence the immune response through nutrition and leads to the working hypothesis that dietary components could be capable of impacting the immune response to a respiratory disease infection.

The overall objective of the research undertaken as part of this program was first to determine the impact of MCFA on viral replication of PRRSV. The first objective was to evaluate the effect of individual MCFA on PRRSV replication in vitro using MARC-145 cells. The second objective was to expand on the knowledge gained in experiment 1 and evaluate the effect of MCFA combinations on PRRSV replication in MARC-145 cells. MCFA combinations were determined by performance of individual MCFA in phase 1. The third objective was to

evaluate the effect of a combination of MCFA delivered through feed in a nursery pig PRRSV

challenge study.

1.6   Literature Cited

Abaidullah, M., S. Peng, M. Kamran, X. Song, and Z. Yin. 2019. Current Findings on Gut Microbiota Mediated Immune Modulation against Viral Diseases in Chicken. Viruses. 11:681. doi:10.3390/v11080681. Available from: www.mdpi.com/journal/viruses

Ahlman, H., and O. Nilsson. 2001. The gut as the largest endocrine organ in the body.

Albina, E., C. Carrat, and B. Charley. 1998. Interferon-α response to swine arterivirus (PoAV), the porcine reproductive and respiratory syndrome virus. J. Interf. Cytokine Res. 18:485–490. doi:10.1089/jir.1998.18.485.

Amadori, M., and E. Razzuoli. 2014. Immune Control of PRRS: Lessons to be Learned and Possible Ways Forward. Front. Vet. Sci. 1. doi:10.3389/fvets.2014.00002. Available from: http://journal.frontiersin.org/article/10.3389/fvets.2014.00002/abstract

Ambagala, A. P. N., J. C. Solheim, and S. Srikumaran. 2005. Viral interference with MHC class I antigen presentation pathway: The battle continues. Vet. Immunol. Immunopathol. 107. doi:10.1016/j.vetimm.2005.04.006.

Ansari, I. H., B. Kwon, F. A. Osorio, and A. K. Pattnaik. 2006. Influence of N-Linked Glycosylation of Porcine Reproductive and Respiratory Syndrome Virus GP5 on Virus Infectivity, Antigenicity, and Ability To Induce Neutralizing Antibodies. J. Virol. 80:3994–4004. doi:10.1128/JVI.80.8.3994-4004.2006. Available from: https://jvi.asm.org/content/80/8/3994

Assimakopoulos, S. F. 2011. Enterocytes' tight junctions: From molecules to diseases. World J. Gastrointest. Pathophysiol. 2:123. doi:10.4291/wjgp.v2.i6.123. Available from: http://www.wjgnet.com/2150-5330/full/v2/i6/123.htm

De Baere, M. I., H. Van Gorp, P. L. Delputte, and H. J. Nauwynck. 2012. Interaction of the European genotype porcine reproductive and respiratory syndrome virus (PRRSV) with sialoadhesin (CD169/Siglec-1) inhibits alveolar macrophage phagocytosis. Vet. Res. 43:47. doi:10.1186/1297-9716-43-47. Available from: http://www.veterinaryresearch.org/content/43/1/47

Benfield, D. A., E. Nelson, J. E. Collins, L. Harris, S. M. Goyal, D. Robison, W. T. Christianson, R. B. Morrison, D. Gorcyca, and D. Chladek. 1992. Characterization of Swine Infertility and Respiratory Syndrome (SIRS) Virus (Isolate ATCC VR-2332). J. Vet. Diagnostic Investig. 4:127–133. doi:10.1177/104063879200400202. Available from: http://journals.sagepub.com/doi/10.1177/104063879200400202

Biebricher, C. K., and M. Eigen. What Is a Quasispecies? In: Quasispecies: Concept and Implications for Virology. Springer-Verlag, Berlin/Heidelberg. p. 1–31. Available from: http://link.springer.com/10.1007/3-540-26397-7_1

Binns, R. M., and R. Pabst. 1994. Lymphoid tissue structure and lymphocyte trafficking in the pig. Vet. Immunol. Immunopathol. 43. doi:10.1016/0165-2427(94)90123-6.

Bogunovic, M., F. Ginhoux, J. Helft, L. Shang, D. Hashimoto, M. Greter, K. Liu, C. Jakubzick, M. A. Ingersoll, M. Leboeuf, E. R. Stanley, M. Nussenzweig, S. A. Lira, G. J. Randolph, and M. Merad. 2009. Origin of the Lamina Propria Dendritic Cell Network. Immunity. 31. doi:10.1016/j.immuni.2009.08.010.

Brar, M. S., M. Shi, R. K.-H. Hui, and F. C.-C. Leung. 2014. Genomic Evolution of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Isolates Revealed by Deep Sequencing. PLoS One. 9. doi:10.1371/journal.pone.0088807.

Van Breedam, W., P. L. Delputte, H. Van Gorp, G. Misinzo, N. Vanderheijden, X. Duan, and H. J. Nauwynck. 2010. Porcine reproductive and respiratory syndrome virus entry into the porcine macrophage. J. Gen. Virol. 91:1659–1667. doi:10.1099/vir.0.020503-0. Available from: https://www.microbiologyresearch.org/content/journal/jgv/10.1099/vir.0.020503-0

Brinkmann, V. 2004. Neutrophil Extracellular Traps Kill Bacteria. Science (80-.). 303:1532–1535. doi:10.1126/science.1092385. Available from: https://www.sciencemag.org/lookup/doi/10.1126/science.1092385

Briscoe, C. P., M. Tadayyon, J. L. Andrews, W. G. Benson, J. K. Chambers, M. M. Eilert, C. Ellis, N. A. Elshourbagy, A. S. Goetz, D. T. Minnick, P. R. Murdock, H. R. Sauls, U. Shabon, L. D. Spinage, J. C. Strum, P. G. Szekeres, K. B. Tan, J. M. Way, D. M. Ignar, S. Wilson, and A. I. Muir. 2003. The Orphan G Protein-coupled Receptor GPR40 Is Activated by Medium and Long Chain Fatty Acids. J. Biol. Chem. 278:11303–11311. doi:10.1074/jbc.M211495200. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925819324147

Brockmeier, S. L., P. G. Halbur, and E. L. Thacker. 2014. Porcine Respiratory Disease Complex. In: Polymicrobial Diseases. ASM Press, Washington, DC, USA. p. 231–258. Available from: http://doi.wiley.com/10.1128/9781555817947.ch13

Budden, K. F., S. L. Gellatly, D. L. A. Wood, M. A. Cooper, M. Morrison, P. Hugenholtz, and P. M. Hansbro. 2017. Emerging pathogenic links between microbiota and the gut–lung axis. Nat. Rev. Microbiol. 15:55–63. doi:10.1038/nrmicro.2016.142. Available from: http://www.nature.com/articles/nrmicro.2016.142

Butler, J. E., K. M. Lager, W. Golde, K. S. Faaberg, M. Sinkora, C. Loving, and Y. I. Zhang. 2014. Porcine reproductive and respiratory syndrome (PRRS): an immune dysregulatory pandemic. Immunol. Res. 59:81–108. doi:10.1007/s12026-014-8549-5. Available from: http://link.springer.com/10.1007/s12026-014-8549-5

Calder, P. C. 2008. The relationship between the fatty acid composition of immune cells and their function. doi:10.1016/j.plefa.2008.09.016. Available from: www.elsevier.com/locate/plefa

Camilleri, M. 2019. Leaky gut: mechanisms, measurement and clinical implications in humans. Gut. 68:1516–1526. doi:10.1136/gutjnl-2019-318427. Available from: https://gut.bmj.com/lookup/doi/10.1136/gutjnl-2019-318427

Castro, G. A., and C. J. Arntzen. 1993. Immunophysiology of the gut: a research frontier for integrative studies of the common mucosal immune system. Am. J. Physiol. Liver Physiol. 265:G599–G610. doi:10.1152/ajpgi.1993.265.4.G599. Available from: https://www.physiology.org/doi/10.1152/ajpgi.1993.265.4.G599

Cesta, M. F. 2006. Normal Structure, Function, and Histology of Mucosa-Associated Lymphoid Tissue. Toxicol. Pathol. 34:599–608. doi:10.1080/01926230600865531. Available from: http://journals.sagepub.com/doi/10.1080/01926230600865531

Chang, C.-C., K.-J. Yoon, J. J. Zimmerman, K. M. Harmon, P. M. Dixon, C. M. T. Dvorak, and M. P. Murtaugh. 2002. Evolution of Porcine Reproductive and Respiratory Syndrome Virus during Sequential Passages in Pigs. J. Virol. 76:4750–4763. doi:10.1128/JVI.76.10.4750-4763.2002. Available from: http://jvi.asm.org/

Chaung, H.-C., C.-W. Chen, B.-L. Hsieh, and W.-B. Chung. 2010. Toll-like receptor expressions in porcine alveolar macrophages and dendritic cells in responding to poly IC stimulation and porcine reproductive and respiratory syndrome virus (PRRSV) infection. Comp. Immunol. Microbiol. Infect. Dis. 33:197–213. doi:10.1016/j.cimid.2008.10.001. Available from: https://linkinghub.elsevier.com/retrieve/pii/S014795710800057X

Chen, C.-J., G.-H. Wu, R.-L. Kuo, and S.-R. Shih. 2017. Role of the intestinal microbiota in the immunomodulation of influenza virus infection. Microbes Infect. 19. doi:10.1016/j.micinf.2017.09.002.

Chen, Z., S. P. Graham, B. Lamp, S. Montaner-Tarbes, L. Fraile, H. A. Del Portillo, and M. Montoya. 2019. Key Gaps in the Knowledge of the Porcine Respiratory Reproductive Syndrome Virus (PRRSV). Front. Vet. Sci. | www.frontiersin.org. 6:38. doi:10.3389/fvets.2019.00038. Available from: www.frontiersin.org

Cochrane, R. A., A. R. Huss, G. C. Aldrich, C. R. Stark, and C. K. Jones. 2016. Evaluating Chemical Mitigation of Salmonella Typhimurium ATCC 14028 in Animal Feed Ingredients. J. Food Prot. 79:672–676. doi:10.4315/0362-028X.JFP-15-320. Available from: https://meridian.allenpress.com/jfp/article/79/4/672/174449/Evaluating-Chemical-Mitigation-of-Salmonella

Darwich, L., I. Díaz, and E. Mateu. 2010. Certainties, doubts and hypotheses in porcine reproductive and respiratory syndrome virus immunobiology. Virus Res. 154:123–132. doi:10.1016/j.virusres.2010.07.017.

Decuypere, J. A., and N. A. Dierick. 2003. The combined use of triacylglycerols containing medium-chain fatty acids and exogenous lipolytic enzymes as an alternative to in-feed antibiotics in piglets: concept, possibilities and limitations. An overview. Nutr. Res. Rev. 16:193–210. doi:10.1079/NRR200369. Available from: https://www.cambridge.org/core/product/identifier/S0954422403000143/type/journal_article

Dee, S. A., F. V. Bauermann, M. C. Niederwerder, A. Singrey, T. Clement, M. de Lima, C. Long, G. Patterson, M. A. Sheahan, A. M. M. Stoian, V. Petrovan, C. K. Jones, J. De Jong, J. Ji, G. D. Spronk, L. Minion, J. Christopher-Hennings, J. J. Zimmerman, R. R. R. Rowland, E. Nelson, P. Sundberg, and D. G. Diel. 2018. Survival of viral pathogens in animal feed ingredients under transboundary shipping models. Y. Cao, editor. PLoS One. 13:e0194509. doi:10.1371/journal.pone.0194509. Available from: https://dx.plos.org/10.1371/journal.pone.0194509

Dee, S. A., M. C. Niederwerder, R. Edler, D. Hanson, A. Singrey, R. Cochrane, G. Spronk, and E. Nelson. 2020. An evaluation of additives for mitigating the risk of virus-contaminated feed using an ice-block challenge model. Transbound. Emerg. Dis. doi:10.1111/tbed.13749.

Dee, S., L. Batista, J. Deen, and C. Pijoan. 2005. Evaluation of an air-filtration system for preventing aerosol transmission of Porcine reproductive and respiratory syndrome virus.

Dee, S., T. Clement, A. Schelkopf, J. Nerem, D. Knudsen, J. Christopher-Hennings, and E. Nelson. 2014. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naïve pigs following consumption via natural feeding behavior: proof of concept. BMC Vet. Res. 10:176. doi:10.1186/s12917-014-0176-9. Available from: http://bmcvetres.biomedcentral.com/articles/10.1186/s12917-014-0176-9

Dee, S., C. Neill, A. Singrey, T. Clement, R. Cochrane, C. Jones, G. Patterson, G. Spronk, J. Christopher-Hennings, and E. Nelson. 2016. Modeling the transboundary risk of feed ingredients contaminated with porcine epidemic diarrhea virus. BMC Vet. Res. 12:51. doi:10.1186/s12917-016-0674-z. Available from: http://www.biomedcentral.com/1746-6148/12/51

Delputte, P. L., P. Meerts, S. Costers, and H. J. Nauwynck. 2004. Effect of virus-specific antibodies on attachment, internalization and infection of porcine reproductive and respiratory syndrome virus in primary macrophages. Vet. Immunol. Immunopathol. 102:179–188. doi:10.1016/j.vetimm.2004.09.007. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165242704002387

Delputte, P. L., N. Vanderheijden, H. J. Nauwynck, and M. B. Pensaert. 2002. Involvement of the Matrix Protein in Attachment of Porcine Reproductive and Respiratory Syndrome Virus to a Heparinlike Receptor on Porcine Alveolar Macrophages. J. Virol. 76:4312–4320. doi:10.1128/JVI.76.9.4312-4320.2002. Available from: http://jvi.asm.org/

Denyer, M. S., T. E. Wileman, C. M. A. Stirling, B. Zuber, and H.-H. Takamatsu. 2006. Perforin expression can define CD8 positive lymphocyte subsets in pigs allowing phenotypic and functional analysis of Natural Killer, Cytotoxic T, Natural Killer T and MHC un-restricted cytotoxic T-cells. Vet. Immunol. Immunopathol. 110:279–292. doi:10.1016/j.vetimm.2005.10.005. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165242705003119

Desbois, A. P., and V. J. Smith. 2010. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl. Microbiol. Biotechnol. 85:1629–1642. doi:10.1007/s00253-009-2355-3. Available from: http://link.springer.com/10.1007/s00253-009-2355-3

Dhakal, J., and C. G. Aldrich. 2020. Use of medium chain fatty acids to mitigate salmonella typhimurium (ATCC 14028) on dry pet food kibbles. J. Food Prot. 83:1505–1511. doi:10.4315/JFP-20-114.

Díaz, I., L. Darwich, G. Pappaterra, J. Pujols, and E. Mateu. 2005. Immune responses of pigs after experimental infection with a European strain of Porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 86:1943–1951. doi:10.1099/vir.0.80959-0. Available from: https://www.microbiologyresearch.org/content/journal/jgv/10.1099/vir.0.80959-0

Díaz, I., L. Darwich, G. Pappaterra, J. Pujols, and E. Mateu. 2006. Different European-type vaccines against porcine reproductive and respiratory syndrome virus have different immunological properties and confer different protection to pigs. Virology. 351:249–259. doi:10.1016/j.virol.2006.03.046. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0042682206002364

Dierick, N., J. Decuypere, and I. Degeyter. 2003. The combined use of whole cuphea seeds containing medium chain fatty acids and an exogenous lipase in piglet nutrition. Arch. Anim. Nutr. 57:49–63. doi:10.1080/0003942031000086626. Available from: http://www.tandfonline.com/doi/abs/10.1080/0003942031000086626

Drake, J. W., and J. J. Holland. 1999. Mutation rates among RNA viruses. Available from: www.pnas.org

Fang, Y., and E. J. Snijder. 2010. The PRRSV replicase: Exploring the multifunctionality of an intriguing set of nonstructural proteins. Virus Res. 154:61–76. doi:10.1016/j.virusres.2010.07.030.

Fischer, C. L., D. R. Drake, D. V Dawson, D. R. Blanchette, K. A. Brogden, and P. W. Wertz. 2011. Antibacterial Activity of Sphingoid Bases and Fatty Acids against Gram-Positive and Gram-Negative Bacteria Downloaded from. doi:10.1128/AAC.05151-11. Available from: http://aac.asm.org/.http://aac.asm.org/

Furness, J. B., W. A. A. Kunze, and N. Clerc. 1999. II. The intestine as a sensory organ: neural, endocrine, and immune responses. Am. J. Physiol. Liver Physiol. 277:G922–G928. doi:10.1152/ajpgi.1999.277.5.G922. Available from: https://www.physiology.org/doi/10.1152/ajpgi.1999.277.5.G922

Furness, J. B., L. R. Rivera, H. J. Cho, D. M. Bravo, and B. Callaghan. 2013. The gut as a sensory organ. Nat. Rev. Gastroenterol. Hepatol. 10:729–740. doi:10.1038/nrgastro.2013.180. Available from: https://www.nature.com/articles/nrgastro.2013.180

Ge, Y., X. Wang, Y. Guo, Junting Yan, A. Abuduwaili, K. Aximujiang, Jie Yan, and M. Wu. 2021. Gut microbiota influence tumor development and Alter interactions with the human immune system. J. Exp. Clin. Cancer Res. 40:42. doi:10.1186/s13046-021-01845-6. Available from: https://jeccr.biomedcentral.com/articles/10.1186/s13046-021-01845-6

Gebert, A., and R. Pabst. 1999. M cells at locations outside the gut. Semin. Immunol. 11:165–170. doi:10.1006/smim.1999.0172. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1044532399901720

Geissmann, F., S. Gordon, D. A. Hume, A. M. Mowat, and G. J. Randolph. 2010. Unravelling mononuclear phagocyte heterogeneity. Nat. Rev. Immunol. 10:453–460. doi:10.1038/nri2784. Available from: http://www.nature.com/articles/nri2784

Genini, S., P. L. Delputte, R. Malinverni, M. Cecere, A. Stella, H. J. Nauwynck, and E. Giuffra. 2008. Genome-wide transcriptional response of primary alveolar macrophages following infection with porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 89:2550–2564. doi:10.1099/vir.0.2008/003244-0. Available from: https://www.microbiologyresearch.org/content/journal/jgv/10.1099/vir.0.2008/003244-0

Gilbert, M. S., N. Ijssennagger, A. K. Kies, and S. W. C. Van Mil. 2018. pigs, and poultry. Perspect. Metabolomics Physiol. Syst. 315:159–170. doi:10.1152/ajpgi.00319.2017.-The. Available from: http://www.ajpgi.org

Guilliams, M., I. De Kleer, S. Henri, S. Post, L. Vanhoutte, S. De Prijck, K. Deswarte, B. Malissen, H. Hammad, and B. N. Lambrecht. 2013. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. J. Exp. Med. 210. doi:10.1084/jem.20131199.

Haddad, J. J., N. E. Saadé, and B. Safieh-Garabedian. 2002. Cytokines and neuro–immune–endocrine interactions: a role for the hypothalamic–pituitary–adrenal revolving axis. J. Neuroimmunol. 133:1–19. doi:10.1016/S0165-5728(02)00357-0. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165572802003570

Haghikia, A., S. Jörg, A. Duscha, J. Berg, A. Manzel, A. Waschbisch, A. Hammer, D.-H. Lee, C. May, N. Wilck, A. Balogh, A. I. Ostermann, N. H. Schebb, D. A. Akkad, D. A. Grohme, M. Kleinewietfeld, S. Kempa, J. Thöne, S. Demir, D. N. Müller, R. Gold, and R. A. Linker. 2015. Dietary Fatty Acids Directly Impact Central Nervous System Autoimmunity via the Small Intestine. Immunity. 43:817–829. doi:10.1016/j.immuni.2015.09.007. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1074761315003921

Heo, J. M., J. C. Kim, J. Yoo, and J. R. Pluske. 2015. A between-experiment analysis of relationships linking dietary protein intake and post-weaning diarrhea in weanling pigs under conditions of experimental infection with an enterotoxigenic strain of Escherichia coli. Anim. Sci. J. 86:286–293. doi:10.1111/asj.12275. Available from: http://doi.wiley.com/10.1111/asj.12275

Hermans, D., and M. De. 2014. Reaching genetic potential with medium chain fatty acids (MCFAs). Int. Poult. Prod. 22:7.

Hill, G. M., G. L. Cromwell, T. D. Crenshaw, C. R. Dove, R. C. Ewan, D. A. Knabe, A. J. Lewis, G. W. Libal, D. C. Mahan, G. C. Shurson, L. L. Southern, and T. L. Veum. 2000. Growth promotion effects and plasma changes from feeding high dietary concentrations of zinc and copper to weanling pigs (regional study). J. Anim. Sci. 78:1010. doi:10.2527/2000.7841010x. Available from: https://academic.oup.com/jas/article/78/4/1010-1016/4668475

Højberg, O., N. Canibe, H. D. Poulsen, M. S. Hedemann, and B. B. Jensen. 2005. Influence of Dietary Zinc Oxide and Copper Sulfate on the Gastrointestinal Ecosystem in Newly Weaned Piglets. Appl. Environ. Microbiol. 71:2267–2277. doi:10.1128/AEM.71.5.2267-2277.2005. Available from: https://aem.asm.org/content/71/5/2267

Hollis, G. R., S. D. Carter, T. R. Cline, T. D. Crenshaw, G. L. Cromwell, G. M. Hill, S. W. Kim, A. J. Lewis, D. C. Mahan, P. S. Miller, H. H. Stein, and T. L. Veum. 2005. Effects of replacing pharmacological levels of dietary zinc oxide with lower dietary levels of various organic zinc sources for weanling pigs1,2. J. Anim. Sci. 83:2123–2129. doi:10.2527/2005.8392123x. Available from: https://academic.oup.com/jas/article/83/9/2123/4790848

Holtkamp, D. J., J. B. Kliebenstein, E. J. Neumann, J. J. Zimmerman, H. F. Rotto, T. K. Yoder, C. Wang, P. E. Yeske, C. L. Mowrer, and C. A. Haley. 2013. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. Available from: http://www.aasv.org/shap.html.

Holzer, P. 2011. TRP Channels in the Digestive System. Curr. Pharm. Biotechnol. 12:24–34. doi:10.2174/138920111793937862. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1389-2010&volume=12&issue=1&spage=24

Huang, Q., D. Feng, K. Liu, P. Wang, H. Xiao, Y. Wang, S. Zhang, and Z. Liu. 2014. A medium-chain fatty acid receptor Gpr84 in zebrafish: Expression pattern and roles in immune regulation. Dev. Comp. Immunol. 45:252–258. doi:10.1016/j.dci.2014.03.017. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0145305X14000858

Hume, D. A. 2008. Differentiation and heterogeneity in the mononuclear phagocyte system. Mucosal Immunol. 1:432–441. doi:10.1038/mi.2008.36. Available from: http://www.nature.com/articles/mi200836

Ishimaru, Y., and H. Matsunami. 2009. CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE. J Dent Res. 88:212–218. doi:10.1177/0022034508330212.

Janssen, S., and I. Depoortere. 2013. Nutrient sensing in the gut: new roads to therapeutics? Trends Endocrinol. Metab. 24:92–100. doi:10.1016/j.tem.2012.11.006. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1043276012002160

Jiang, Y., G. Li, L. Yu, L. Li, Y. Zhang, Y. Zhou, W. Tong, C. Liu, F. Gao, and G. Tong. 2020. Genetic Diversity of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) From 1996 to 2017 in China. Front. Microbiol. 11. doi:10.3389/fmicb.2020.00618. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2020.00618/full

Johansson, M. E. V., M. Phillipson, J. Petersson, A. Velcich, L. Holm, and G. C. Hansson. 2008. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc. Natl. Acad. Sci. 105:15064–15069. doi:10.1073/pnas.0803124105. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0803124105

Kamada, N., and G. Núñez. 2014. Regulation of the Immune System by the Resident Intestinal Bacteria. Gastroenterology. 146:1477–1488. doi:10.1053/j.gastro.2014.01.060. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0016508514001516

Karniychuk, U. U., and H. J. Nauwynck. 2013. Pathogenesis and prevention of placental and transplacental porcine reproductive and respiratory syndrome virus infection. Vet. Res. 44. doi:10.1186/1297-9716-44-95.

Karniychuk, U. U., D. Saha, M. Geldhof, M. Vanhee, P. Cornillie, W. Van den Broeck, and H. J. Nauwynck. 2011. Porcine reproductive and respiratory syndrome virus (PRRSV) causes apoptosis during its replication in fetal implantation sites. Microb. Pathog. 51. doi:10.1016/j.micpath.2011.04.001.

Kimman, T. G., L. A. Cornelissen, R. J. Moormann, J. M. J. Rebel, and N. Stockhofe-Zurwieden. 2009. Challenges for porcine reproductive and respiratory syndrome virus (PRRSV) vaccinology. Vaccine. 27:3704–3718. doi:10.1016/j.vaccine.2009.04.022.

Kobayashi, B. Y. M., L. Fitz, M. Ryan, R. M. Hewick, S. C. Clark, S. Chan, R. Loudon, F. Sherman, B. Perussia, and G. Trinchieri. 1989. IDENTIFICATION AND PURIFICATION OF NATURAL KILLER CELL STIMULATORY FACTOR (NKSF), A CYTOKINE WITH MULTIPLE BIOLOGIC EFFECTS ON HUMAN LYMPHOCYTES Human B lymphoblastoid cell lines facilitate the growth in vitro of human NK cells and of T cell clones (1-. J. Exp. Med. 170:827–845.

Komatsu, T., Z. Bi, and C. S. Reiss "b'''"." 1996. Interferon-y induced type I nitric oxide synthase activity inhibits viral replication in neurons.

Kumar, H., T. Kawai, and S. Akira. 2011. Pathogen Recognition by the Innate Immune System. Int. Rev. Immunol. 30:16–34. doi:10.3109/08830185.2010.529976. Available from: http://www.tandfonline.com/doi/full/10.3109/08830185.2010.529976

Kumar, V., and A. Sharma. 2010. Neutrophils: Cinderella of innate immune system. Int. Immunopharmacol. 10:1325–1334. doi:10.1016/j.intimp.2010.08.012. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1567576910002663

Kvisgaard, L. K., C. S. Kristensen, P. Ryt-Hansen, K. Pedersen, T. Stadejek, R. Trebbien, L. O. Andresen, and L. E. Larsen. 2020. A recombination between two Type 1 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV-1) vaccine strains has caused severe outbreaks in Danish pigs. Transbound. Emerg. Dis. 67:1786–1796. doi:10.1111/tbed.13555. Available from: https://onlinelibrary.wiley.com/doi/10.1111/tbed.13555

Kwon, B., I. H. Ansari, A. K. Pattnaik, and F. A. Osorio. 2008. Immune responses in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV). Virology. 380:371–378. doi:10.1016/S0165-2427(97)00134-7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S004268220800490X

Lager, K. M., and P. G. Halbur. 1996. Gross and Microscopic Lesions in Porcine Fetuses Infected with Porcine Reproductive and Respiratory Syndrome Virus. J. Vet. Diagnostic Investig. 8. doi:10.1177/104063879600800301.

Lawson, S. R., K. D. Rossow, J. E. Collins, D. A. Benfield, and R. R. . Rowland. 1997. Porcine reproductive and respiratory syndrome virus infection of gnotobiotic pigs: sites of virus replication and co-localization with MAC-387 staining at 21 days post-infection. Virus Res. 51. doi:10.1016/S0168-1702(97)00086-5.

Lee, E. J., E. Ko, J. Lee, S. Rho, S. Ko, M. K. Shin, B. Il Min, M. C. Hong, S. Y. Kim, and H. Bae. 2004. Ginsenoside Rg1 enhances CD4+ T-cell activities and modulates Th1/Th2 differentiation. Int. Immunopharmacol. 4:235–244. doi:10.1016/j.intimp.2003.12.007.

Lee, S.-M., S. K. Schommer, and S. B. Kleiboeker. 2004. Porcine reproductive and respiratory syndrome virus field isolates differ in in vitro interferon phenotypes. Vet. Immunol. Immunopathol. 102. doi:10.1016/j.vetimm.2004.09.009.

Lee, Y. J., and C. Lee. 2010. Deletion of the cytoplasmic domain of CD163 enhances porcine reproductive and respiratory syndrome virus replication. Arch. Virol. 155:1319–1323. doi:10.1007/s00705-010-0699-8. Available from: http://link.springer.com/10.1007/s00705-010-0699-8

Lee, Y. J., C.-K. Park, E. Nam, S.-H. Kim, O.-S. Lee, D. S. Lee, and C. Lee. 2010. Generation of a porcine alveolar macrophage cell line for the growth of porcine reproductive and respiratory syndrome virus. J. Virol. Methods. 163:410–415. doi:10.1016/j.jviromet.2009.11.003. Available from: https://linkinghub.elsevier.com/retrieve/pii/S016609340900473X

Lerner, A. B., R. A. Cochrane, J. T. Gebhardt, S. S. Dritz, C. K. Jones, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. Bai, E. Porter, J. Anderson, P. C. Gauger, D. R. Magstadt, J. Zhang, B. Bass, T. Karnezos, B. de Rodas, and J. C. Woodworth. 2020. Effects of medium chain fatty acids as a mitigation or prevention strategy against porcine epidemic diarrhea virus in swine feed. J. Anim. Sci. 98. doi:10.1093/jas/skaa159. Available from: https://academic.oup.com/jas/article/doi/10.1093/jas/skaa159/5843591

Li, J., S. Wang, C. Li, C. Wang, Yonggang Liu, G. Wang, X. He, L. Hu, Yuanyuan Liu, M. Cui, C. Bi, Z. Shao, X. Wang, T. Xiong, X. Cai, L. Huang, and C. Weng. 2017. Secondary Haemophilus parasuis infection enhances highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) infection-mediated inflammatory responses. doi:10.1016/j.vetmic.2017.03.035. Available from: http://dx.doi.org/10.1016/j.vetmic.2017.03.035

Liu, B., W. Wang, X. Zhu, X. Sun, J. Xiao, D. Li, Y. Cui, C. Wang, and Y. Shi. 2018. Response of Gut Microbiota to Dietary Fiber and Metabolic Interaction With SCFAs in Piglets. Front. Microbiol. 9. doi:10.3389/fmicb.2018.02344. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2018.02344/full

Liu, C.-H., H.-C. Chaung, H. Chang, Y.-T. Peng, and W.-B. Chung. 2009. Expression of Toll-like receptor mRNA and cytokines in pigs infected with porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 136:266–276. doi:10.1016/j.vetmic.2008.11.016. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378113508005464

Loving, C. L., S. L. Brockmeier, and R. E. Sacco. 2007. Differential type I interferon activation and susceptibility of dendritic cell populations to porcine arterivirus. Immunology. 120:217–229. doi:10.1111/j.1365-2567.2006.02493.x. Available from: http://doi.wiley.com/10.1111/j.1365-2567.2006.02493.x

Magyar, A., R. Mihalik, and I. Oláh. 1995. The surface phenotype of swine blood and tissue eosinophil granulocytes. Vet. Immunol. Immunopathol. 47:273–281. doi:10.1016/0165-2427(94)05369-4. Available from: https://linkinghub.elsevier.com/retrieve/pii/0165242794053694

Mair, K. H., C. Sedlak, T. Käser, A. Pasternak, B. Levast, W. Gerner, A. Saalmüller, A. Summerfield, V. Gerdts, H. L. Wilson, and F. Meurens. 2014. The porcine innate immune system: An update. Dev. Comp. Immunol. 45:321–343. doi:10.1016/j.dci.2014.03.022.

Masure, D., J. Vlaminck, T. Wang, K. Chiers, W. Van den Broeck, J. Vercruysse, and P. Geldhof. 2013. A Role for Eosinophils in the Intestinal Immunity against Infective Ascaris suum Larvae. E. Mitre, editor. PLoS Negl. Trop. Dis. 7:e2138. doi:10.1371/journal.pntd.0002138. Available from: https://dx.plos.org/10.1371/journal.pntd.0002138

Mateu, E., and I. Diaz. 2008. The challenge of PRRS immunology. Vet. J. 177:345–351. doi:10.1016/j.tvjl.2007.05.022.

McDonald, G. B., D. R. Saunders, M. Weidman, and L. Fisher. 1980. Portal venous transport of long-chain fatty acids absorbed from rat intestine. Am. J. Physiol. Liver Physiol. 239. doi:10.1152/ajpgi.1980.239.3.G141.

Meier, W. A., J. Galeota, F. A. Osorio, R. J. Husmann, W. M. Schnitzlein, and F. A. Zuckermann. 2003. Gradual development of the interferon-γ response of swine to porcine reproductive and respiratory syndrome virus infection or vaccination. Virology. 309:18–31. doi:10.1016/S0042-6822(03)00009-6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0042682203000096

Merad, M., P. Sathe, J. Helft, J. Miller, and A. Mortha. 2013. The Dendritic Cell Lineage: Ontogeny and Function of Dendritic Cells and Their Subsets in the Steady State and the Inflamed Setting. Annu. Rev. Immunol. 31:563–604. doi:10.1146/annurev-immunol-020711-074950. Available from: http://www.annualreviews.org/doi/10.1146/annurev-immunol-020711-074950

Miller, L. C., G. P. Harhay, K. M. Lager, T. P. L. Smith, and J. D. Neill. 2008. Effect of Porcine Reproductive and Respiratory Syndrome Virus on Porcine Alveolar Macrophage Function as Determined Using Serial Analysis of Gene Expression (SAGE). In: Animal Genomics for Animal Health. KARGER, Basel. p. 169–174. Available from: https://www.karger.com/Article/FullText/317157

Miller, L. C., K. M. Lager, and M. E. Kehrli. 2009. Role of Toll-Like Receptors in Activation of Porcine Alveolar Macrophages by Porcine Reproductive and Respiratory Syndrome Virus. Clin. Vaccine Immunol. 16:360–365. doi:10.1128/CVI.00269-08. Available from: https://cvi.asm.org/content/16/3/360

Monte B. McCaw, D. P. McCaw MB. Effect of reducing crossfostering at birth on piglet mortality and performance during an acute outbreak of porcine reproductive and respiratory syndrome. . Swine Heal. Prod. 2000;8(1)15-21.

Murtaugh, M. P., and M. Genzow. 2011. Immunological solutions for treatment and prevention of porcine reproductive and respiratory syndrome (PRRS). Vaccine. 29:8192–8204. doi:10.1016/j.vaccine.2011.09.013.

Murtaugh, M. P., T. Stadejek, J. E. Abrahante, T. T. Y. Lam, and F. C. C. Leung. 2010. The ever-expanding diversity of porcine reproductive and respiratory syndrome virus. Virus Res. 154:18–30. doi:10.1016/j.virusres.2010.08.015.

Nabuurs, M. J. A., A. Hoogendoorn, E. J. Van Der Molen, and A. L. M. Van Osta. 1993. Villus height and crypt depth in weaned and unweaned pigs, reared under various circumstances in the Netherlands. Res. Vet. Sci. 55:78–84. doi:10.1016/0034-5288(93)90038-H. Available from: https://linkinghub.elsevier.com/retrieve/pii/003452889390038H

Nazki, S., A. Khatun, C.-G. Jeong, S. ul S. Mattoo, S. Gu, S.-I. Lee, S.-C. Kim, J.-H. Park, M.-S. Yang, B. Kim, C.-K. Park, S.-M. Lee, and W.-I. Kim. 2020. Evaluation of local and systemic immune responses in pigs experimentally challenged with porcine reproductive and respiratory syndrome virus. Vet. Res. 51. doi:10.1186/s13567-020-00789-7.

Nelson, E. A., J. Christopher-Hennings, and D. A. Benfield. 1994. Serum Immune Responses to the Proteins of Porcine Reproductive and Respiratory Syndrome (PRRS) Virus. J. Vet. Diagnostic Investig. 6:410–415. doi:10.1177/104063879400600402.

Niederwerder, M. C., S. Dee, D. G. Diel, A. M. M. Stoian, L. A. Constance, M. Olcha, V. Petrovan, G. Patterson, A. G. Cino-Ozuna, and R. R. R. Rowland. 2020. Mitigating the risk of African swine fever virus in feed with anti-viral chemical additives. Transbound. Emerg. Dis. doi:10.1111/tbed.13699.

Nikitina, E., I. Larionova, E. Choinzonov, and J. Kzhyshkowska. 2018. Molecular Sciences Monocytes and Macrophages as Viral Targets and Reservoirs. doi:10.3390/ijms19092821. Available from: www.mdpi.com/journal/ijms

Noguchi, O., H. Takeuchi, F. Kubota, H. Tsuji, and T. Aoyama. 2002. Larger Diet-Induced Thermogenesis and Less Body Fat Accumulation in Rats Fed Medium-Chain Triacylglycerols than in Those Fed Long-Chain Triacylglycerols. J. Nutr. Sci. Vitaminol. (Tokyo). 48:524–529. doi:10.3177/jnsv.48.524. Available from: http://joi.jlc.jst.go.jp/JST.Journalarchive/jnsv1973/48.524?from=CrossRef

Novakovic, P., J. C. S. Harding, A. N. Al-Dissi, A. Ladinig, and S. E. Detmer. 2016. Pathologic Evaluation of Type 2 Porcine Reproductive and Respiratory Syndrome Virus Infection at the Maternal-Fetal Interface of Late Gestation Pregnant Gilts. PLoS One. 11. doi:10.1371/journal.pone.0151198.

Okumura, R., and K. Takeda. 2016. Maintenance of gut homeostasis by the mucosal immune system. Proc. Japan Acad. Ser. B. 92:423–435. doi:10.2183/pjab.92.423. Available from: https://www.jstage.jst.go.jp/article/pjab/92/9/92_PJA9209B-03/_article

Oleksiewicz, M. B., and J. Nielsen. 1999. Effect of porcine reproductive and respiratory syndrome virus (PRRSV) on alveolar lung macrophage survival and function. Vet. Microbiol. 66. doi:10.1016/S0378-1135(98)00309-5.

Ostrowski, M., J. A. Galeota, A. M. Jar, K. B. Platt, F. A. Osorio, and O. J. Lopez. 2002. Identification of Neutralizing and Nonneutralizing Epitopes in the Porcine Reproductive and Respiratory Syndrome Virus GP5 Ectodomain. J. Virol. 76:4241–4250. doi:10.1128/JVI.76.9.4241-4250.2002. Available from: https://jvi.asm.org/content/76/9/4241

Otake, S., S. A. Dee, D. Acvm, K. D. Rossow, J. Deen, D. Abvp, H. Soo Joo, T. W. Molitor, and C. Pijoan. 2002. Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). Available from: http://www.aasv.org/shap.html.

Pang, I. K., and A. Iwasaki. 2011. Inflammasomes as mediators of immunity against influenza virus. Trends Immunol. 32. doi:10.1016/j.it.2010.11.004.

Papamandjaris, A. A., D. E. Macdougall, and P. J. H. Jones. 1998. Medium chain fatty acid metabolism and energy expenditure: Obesity treatment implications. Life Sci. 62. doi:10.1016/S0024-3205(97)01143-0.

Park, C., J. H. Baek, S. H. Cho, J. Jeong, C. Chae, S.-H. You, and S.-H. Cha. 2020. Field porcine reproductive and respiratory syndrome viruses (PRRSV) attenuated by codon pair deoptimization (CPD) in NSP1 protected pigs from heterologous challenge. Virology. 540:172–183. doi:10.1016/j.virol.2019.10.019. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0042682219303083

Park, K.-M., S. J. Lee, H. Yu, J.-Y. Park, H.-S. Jung, K. Kim, C. J. Lee, and P.-S. Chang. 2018. Hydrophilic and lipophilic characteristics of non-fatty acid moieties: significant factors affecting antibacterial activity of lauric acid esters. Food Sci. Biotechnol. 27:401–409. doi:10.1007/s10068-018-0353-x. Available from: http://link.springer.com/10.1007/s10068-018-0353-x

Pasternak, A. O. 2001. Sequence requirements for RNA strand transfer during nidovirus discontinuous subgenomic RNA synthesis. EMBO J. 20:7220–7228. doi:10.1093/emboj/20.24.7220. Available from: http://emboj.embopress.org/cgi/doi/10.1093/emboj/20.24.7220

Patton, J. B., R. R. Rowland, D. Yoo, and K.-O. Chang. 2009. Modulation of CD163 receptor expression and replication of porcine reproductive and respiratory syndrome virus in porcine macrophages. Virus Res. 140:161–171. doi:10.1016/j.virusres.2008.12.002. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168170208004097

Pérez, C., E. Ortuño, N. Gómez, M. García-Briones, B. Álvarez, P. M. De La Riva, F. Alonso, C. Revilla, J. Domínguez, and A. Ezquerra. 2008. Cloning and expression of porcine CD163: Its use for characterization of monoclonal antibodies to porcine CD163 and development of an ELISA to measure soluble CD163 in biological fluids. Spanish J. Agric. Res. 6:59–72. doi:10.5424/sjar/200806s1-374.

Pié, S., J. P. Lallès, F. Blazy, J. Laffitte, B. Sève, and I. P. Oswald. 2004. Weaning Is Associated with an Upregulation of Expression of Inflammatory Cytokines in the Intestine of Piglets. J. Nutr. 134:641–647. doi:10.1093/jn/134.3.641. Available from: https://academic.oup.com/jn/article/134/3/641/4688512

Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. 51:215–236. doi:10.1016/S0301-6226(97)00057-2. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0301622697000572

Rahe, M. C., M. P. Murtaugh, L. Dixon, and S. Graham. 2017. Mechanisms of Adaptive Immunity to Porcine Reproductive and Respiratory Syndrome Virus. doi:10.3390/v9060148. Available from: www.mdpi.com/journal/viruses

Ravindran, V., P. Tancharoenrat, F. Zaefarian, and G. Ravindran. 2016. Fats in poultry nutrition: Digestive physiology and factors influencing their utilisation. Anim. Feed Sci. Technol. 213:1–21. doi:10.1016/j.anifeedsci.2016.01.012. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0377840116300293

Rehfeld, J. F. 2004. A Centenary of Gastrointestinal Endocrinology. Horm. Metab. Res. 36:735–741. doi:10.1055/s-2004-826154. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-2004-826154

Rossi, R., G. Pastorelli, S. Cannata, and C. Corino. 2010. Recent advances in the use of fatty acids as supplements in pig diets: A review. Anim. Feed Sci. Technol. 162:1–11. doi:10.1016/j.anifeedsci.2010.08.013. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0377840110002804

Rossow, K. D. 1998. Porcine Reproductive and Respiratory Syndrome. Vet. Pathol. 35. doi:10.1177/030098589803500101.

Rothkötter, H.-J. 2009. Anatomical particularities of the porcine immune system—A physician's view. Dev. Comp. Immunol. 33:267–272. doi:10.1016/j.dci.2008.06.016. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0145305X08001638

Rowland, R. R. ., S. Lawson, K. Rossow, and D. A. Benfield. 2003. Lymphoid tissue tropism of porcine reproductive and respiratory syndrome virus replication during persistent infection of pigs originally exposed to virus in utero. Vet. Microbiol. 96. doi:10.1016/j.vetmic.2003.07.006.

Salgado, P., J. P. . Freire, M. Mourato, F. Cabral, R. Toullec, and J. . Lallès. 2002. Comparative effects of different legume protein sources in weaned piglets: nutrient digestibility, intestinal morphology and digestive enzymes. Livest. Prod. Sci. 74:191–202. doi:10.1016/S0301-6226(01)00297-4. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0301622601002974

Sánchez, C., N. Doménech, J. Vázquez, F. Alonso, A. Ezquerra, and J. Domínguez. 1999. The porcine 2A10 antigen is homologous to human CD163 and related to macrophage differentiation. J. Immunol. 162:5230–5237.

Sang, Y., C. R. Ross, R. R. R. Rowland, and F. Blecha. 2008. Toll-like Receptor 3 Activation Decreases Porcine Arterivirus Infection. Viral Immunol. 21:303–314. doi:10.1089/vim.2008.0042. Available from: https://www.liebertpub.com/doi/10.1089/vim.2008.0042

Schulz, O., E. Jaensson, E. K. Persson, X. Liu, T. Worbs, W. W. Agace, and O. Pabst. 2009. Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. J. Exp. Med. 206. doi:10.1084/jem.20091925.

Shelton, N. W., M. D. Tokach, J. L. Nelssen, R. D. Goodband, S. S. Dritz, J. M. DeRouchey, and G. M. Hill. 2011. Effects of copper sulfate, tri-basic copper chloride, and zinc oxide on weanling pig performance1. J. Anim. Sci. 89:2440–2451. doi:10.2527/jas.2010-3432. Available from: https://academic.oup.com/jas/article/89/8/2440-2451/4764479

Shishido, S. N., S. Varahan, K. Yuan, X. Li, and S. D. Fleming. 2012. Humoral innate immune response and disease. Clin. Immunol. 144:142–158. doi:10.1016/j.clim.2012.06.002. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1521661612001568

Sinkora, M., and J. Sinkorova. 2014. B Cell Lymphogenesis in Swine Is Located in the Bone Marrow. J. Immunol. 193:5023–5032. doi:10.4049/jimmunol.1401152. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1401152

Šinkora, Šinkora, Řehakova, Šplichal, Yang, Parkhouse, and Trebichavsky. 1998. Prenatal ontogeny of lymphocyte subpopulations in pigs. Immunology. 95:595–603. doi:10.1046/j.1365-2567.1998.00641.x. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2567.1998.00641.x

Sips, M., M. Krykbaeva, T. J. Diefenbach, M. Ghebremichael, B. A. Bowman, A.-S. Dugast, A. W. Boesch, H. Streeck, D. S. Kwon, M. E. Ackerman, T. J. Suscovich, P. Brouckaert, T. W. Schacker, and G. Alter. 2016. Fc receptor-mediated phagocytosis in tissues as a potent mechanism for preventive and therapeutic HIV vaccine strategies. 9. doi:10.1038/mi.2016.12. Available from: www.nature.com/mi

Slifka, M. K., R. Antia, J. K. Whitmire, and R. Ahmed. 1998. Humoral Immunity Due to Long-Lived Plasma Cells. Immunity. 8:363–372. doi:10.1016/S1074-7613(00)80541-5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1074761300805415

Snijder, E. J., and J. J. M. Meulenberg. 1998. Printed in Great Britain The molecular biology of arteriviruses.

Spriggs, M. K., D. E. Hruby, C. R. Maliszewski, D. J. Pickup, J. E. Sims, R. M. L. Buller, and J. VanSlyke. 1992. Vaccinia and cowpox viruses encode a novel secreted interleukin-1-binding protein. Cell. 71. doi:10.1016/0092-8674(92)90273-F.

Sun, M., W. Wu, Z. Liu, and Y. Cong. 2017. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. J. Gastroenterol. 52:1–8. doi:10.1007/s00535-016-1242-9. Available from: http://link.springer.com/10.1007/s00535-016-1242-9

Sun, Y., M. Han, C. Kim, J. G. Calvert, and D. Yoo. 2012. Interplay between Interferon-Mediated Innate Immunity and Porcine Reproductive and Respiratory Syndrome Virus. Viruses. 4:424–446. doi:10.3390/v4040424. Available from: www.mdpi.com/journal/viruses

Suradhat, S., and R. Thanawongnuwech. 2003. Upregulation of interleukin-10 gene expression in the leukocytes of pigs infected with porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 84:2755–2760. doi:10.1099/vir.0.19230-0. Available from: https://www.microbiologyresearch.org/content/journal/jgv/10.1099/vir.0.19230-0

Suzuki, M., S. Takaishi, M. Nagasaki, Y. Onozawa, I. Iino, H. Maeda, T. Komai, and T. Oda. 2013. Medium-chain Fatty Acid-sensing Receptor, GPR84, Is a Proinflammatory Receptor. J. Biol. Chem. 288:10684–10691. doi:10.1074/jbc.M112.420042. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925820672908

Szabo, A., and E. Rajnavolgyi. 2013. Collaboration of Toll-like and RIG-I-like receptors in human dendritic cells: tRIGgering antiviral innate immune responses. Am J Clin Exp Immunol. 2:195–207. Available from: www.ajcei.us/ISSN:2164-7712/AJCEI1309001

Tan, J. K., C. McKenzie, E. Mariño, L. Macia, and C. R. Mackay. 2017. Metabolite-Sensing G Protein–Coupled Receptors—Facilitators of Diet-Related Immune Regulation. Annu. Rev. Immunol. 35:371–402. doi:10.1146/annurev-immunol-051116-052235. Available from: http://www.annualreviews.org/doi/10.1146/annurev-immunol-051116-052235

Thaa, B., B. C. Sinhadri, C. Tielesch, E. Krause, and M. Veit. 2013. Signal Peptide Cleavage from GP5 of PRRSV: A Minor Fraction of Molecules Retains the Decoy Epitope, a Presumed Molecular Cause for Viral Persistence) Signal Peptide Cleavage from GP5 of PRRSV: A Minor Fraction of Molecules Retains the Decoy Epitope, a Presumed Molecular Cause for Viral. Persistence. PLoS ONE. 8:65548. doi:10.1371/journal.pone.0065548. Available from: www.plosone.org

Thanawongnuwech, R., A. Amonsin, A. Tatsanakit, and S. Damrongwatanapokin. 2004. Genetics and geographical variation of porcine reproductive and respiratory syndrome virus (PRRSV) in Thailand. Vet. Microbiol. 101:9–21. doi:10.1016/j.vetmic.2004.03.005.

Thomas, L. L., J. C. Woodworth, M. D. Tokach, S. S. Dritz, J. M. DeRouchey, R. D. Goodband, H. E. Williams, A. R. Hartman, D. J. Mellick, D. M. McKilligan, and A. M. Jones. 2020. Evaluation of different blends of medium-chain fatty acids, lactic acid, and monolaurin on nursery pig growth performance12. Transl. Anim. Sci. 4:548–557. doi:10.1093/tas/txaa024.

Thormar, H., C. E. Isaacs, H. R. Brown, M. R. Barshatzky, and T. Pessolano. 1987. Inactivation of enveloped viruses and killing of cells by fatty acids and monoglycerides. Antimicrob. Agents Chemother. 31:27–31. doi:10.1128/AAC.31.1.27. Available from: http://aac.asm.org/cgi/doi/10.1128/AAC.31.1.27

Tsuzuki, Y., J. Miyazaki, K. Matsuzaki, Y. Okada, R. Hokari, A. Kawaguchi, S. Nagao, K. Itoh, and S. Miura. 2006. Differential modulation in the functions of intestinal dendritic cells by long- and medium-chain fatty acids. J. Gastroenterol. 41:209–216. doi:10.1007/s00535-005-1747-0. Available from: http://link.springer.com/10.1007/s00535-005-1747-0

Turner, J. R. 2009. Complex multicellular organisms interface with their external environments at multiple sites. Nat. Rev. Immunol. doi:10.1038/nri2653. Available from: www.nature.com/reviews/immunol

Uston, P. I., J. F. Jr. Urban, M. Ashraf, C. M. Lee, and F. R. Ampy. 2007. L3L4ES antigen and secretagogues induce histamine release from porcine peripheral blood basophils after Ascaris suum infection. Parasitol. Res. 100:603–611. doi:10.1007/s00436-006-0362-1. Available from: http://link.springer.com/10.1007/s00436-006-0362-1

Vitale, S., S. Picascia, and C. Gianfrani. 2016. The cross-talk between enterocytes and intraepithelial lymphocytes. doi:10.1186/s40348-016-0048-4.

Walk, C. L., P. Wilcock, and E. Magowan. 2015. Evaluation of the effects of pharmacological zinc oxide and phosphorus source on weaned piglet growth performance, plasma minerals and mineral digestibility. Animal. 9:1145–1152. doi:10.1017/S175173111500035X. Available from: https://linkinghub.elsevier.com/retrieve/pii/S175173111500035X

Wang, J., X. Wu, N. Simonavicius, H. Tian, and L. Ling. 2006. Medium-chain fatty acids as ligands for orphan G protein-coupled receptor GPR84. J. Biol. Chem. 281:34457–34464. doi:10.1074/jbc.M608019200.

Wang, N., H. Liang, K. Zen, and L. L. Lenz. 2014. Molecular mechanisms that influence the macrophage M1-M2 polarization balance. doi:10.3389/fimmu.2014.00614. Available from: www.frontiersin.org

Wang, X., M. Eaton, M. Mayer, H. Li, D. He, E. Nelson, and J. Christopher-Hennings. 2007. Porcine reproductive and respiratory syndrome virus productively infects monocyte-derived dendritic cells and compromises their antigen-presenting ability. Arch. Virol. 152:289–303. doi:10.1007/s00705-006-0857-1. Available from: http://link.springer.com/10.1007/s00705-006-0857-1

Welch, S. K. W., and J. G. Calvert. 2010. A brief review of CD163 and its role in PRRSV infection. Virus Res. 154:98–103. doi:10.1016/j.virusres.2010.07.018.

Wells, J. M., R. J. Brummer, M. Derrien, T. T. Macdonald, F. Troost, P. D. Cani, V. Theodorou, J. Dekker, A. Méheust, W. M. De Vos, A. Mercenier, A. Nauta, and C. L. Garcia-Rodenas. 2017. Homeostasis of the gut barrier and potential biomarkers Licensed under Creative Commons Attribution CC-BY 3.0. Am J Physiol Gastrointest Liver Physiol. 312:171–193. doi:10.1152/ajpgi.00048.2015.-The. Available from: http://www.ajpgi.org

Wills, R. W., J. J. Zimmerman, K.-J. Yoon, S. L. Swenson, M. J. McGinley, H. T. Hill, K. B. Platt, J. Christopher-Hennings, and E. A. Nelson. 1997. Porcine reproductive and respiratory syndrome virus: a persistent infection. Vet. Microbiol. 55:231–240. doi:10.1016/S0378-1135(96)01337-5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378113596013375

Windisch, W., K. Schedle, C. Plitzner, and A. Kroismayr. 2008. Use of phytogenic products as feed additives for swine and poultry1. J. Anim. Sci. 86:E140–E148. doi:10.2527/jas.2007-0459. Available from: https://academic.oup.com/jas/article/86/suppl_14/E140/4789896

Wissink, E. H. J., M. V. Kroese, H. A. R. van Wijk, F. A. M. Rijsewijk, J. J. M. Meulenberg, and P. J. M. Rottier. 2005. Envelope Protein Requirements for the Assembly of Infectious Virions of Porcine Reproductive and Respiratory Syndrome Virus. J. Virol. 79:12495–12506. doi:10.1128/JVI.79.19.12495-12506.2005. Available from: https://jvi.asm.org/content/79/19/12495

Wu, G. D., J. Chen, C. Hoffmann, K. Bittinger, Y.-Y. Chen, S. A. Keilbaugh, M. Bewtra, D. Knights, W. A. Walters, R. Knight, R. Sinha, E. Gilroy, K. Gupta, R. Baldassano, L. Nessel, H. Li, F. D. Bushman, and J. D. Lewis. 2011. Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. Science (80-.). 334:105–108. doi:10.1126/science.1208344. Available from: https://www.sciencemag.org/lookup/doi/10.1126/science.1208344

Xiao, C., J. Hsieh, K. Adeli, and G. F. Lewis. 2011. Gut-liver interaction in triglyceride-rich lipoprotein metabolism. Am. J. Physiol. Metab. 301:E429–E446. doi:10.1152/ajpendo.00178.2011. Available from: https://www.physiology.org/doi/10.1152/ajpendo.00178.2011

Xiao, S., J. Jia, D. Mo, Q. Wang, L. Qin, Z. He, X. Zhao, Y. Huang, A. Li, J. Yu, Y. Niu, X. Liu, and Y. Chen. 2010. Understanding PRRSV infection in porcine lung based on genome-wide transcriptome response identified by deep sequencing. PLoS One. 5. doi:10.1371/journal.pone.0011377.

Yoon, B., J. Jackman, E. Valle-González, and N.-J. Cho. 2018. Antibacterial Free Fatty Acids and Monoglycerides: Biological Activities, Experimental Testing, and Therapeutic Applications. Int. J. Mol. Sci. 19:1114. doi:10.3390/ijms19041114. Available from: http://www.mdpi.com/1422-0067/19/4/1114

Yoon, B. K., J. A. Jackman, M. C. Kim, and N.-J. Cho. 2015. Spectrum of Membrane Morphological Responses to Antibacterial Fatty Acids and Related Surfactants. Langmuir. 31:10223–10232. doi:10.1021/acs.langmuir.5b02088. Available from: https://pubs.acs.org/doi/10.1021/acs.langmuir.5b02088

Yoon, I. J., H. S. Joo, S. M. Goyal, and T. W. Molitor. 1994. A modified serum neutralization test for the detection of antibody to porcine reproductive and respiratory syndrome virus in swine sera.

Yoon, K.-J., L.-L. Wu, J. J. Zimmerman, H. T. Hill, and K. B. Platt. 1996. Antibody-Dependent Enhancement (ADE) of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Infection in Pigs. Viral Immunol. 9:51–63. doi:10.1089/vim.1996.9.51. Available from: https://www.liebertpub.com/doi/10.1089/vim.1996.9.51

Yu, D., W. Zhu, and S. Hang. 2019. Effects of Long-Term Dietary Protein Restriction on Intestinal Morphology, Digestive Enzymes, Gut Hormones, and Colonic Microbiota in Pigs. Animals. 9:180. doi:10.3390/ani9040180. Available from: www.mdpi.com/journal/animals

Zeng, Z., S. Zhang, H. Wang, and X. Piao. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. J. Anim. Sci. Biotechnol. 6:7. doi:10.1186/s40104-015-0004-5. Available from: https://jasbsci.biomedcentral.com/articles/10.1186/s40104-015-0004-5

Zentek, J., S. Buchheit-Renko, F. Ferrara, W. Vahjen, A. G. Van Kessel, and R. Pieper. 2011. Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Anim. Health Res. Rev. 12:83–93. doi:10.1017/S1466252311000089.

Zentek, J., S. Buchheit-Renko, K. Männer, R. Pieper, and W. Vahjen. 2012. Intestinal concentrations of free and encapsulated dietary medium-chain fatty acids and effects on gastric microbial ecology and bacterial metabolic products in the digestive tract of piglets. Arch. Anim. Nutr. 66:14–26. doi:10.1080/1745039X.2011.644916. Available from: http://www.tandfonline.com/doi/abs/10.1080/1745039X.2011.644916

Zhao, Y., G. Tian, D. Chen, P. Zheng, J. Yu, J. He, X. Mao, Z. Huang, Y. Luo, J. Luo, and B. Yu. 2020. Dietary protein levels and amino acid supplementation patterns alter the composition and functions of colonic microbiota in pigs. Anim. Nutr. 6:143–151. doi:10.1016/j.aninu.2020.02.005.

Zhou, L., L. Fang, Y. Sun, Y. Su, and W. Zhu. 2016. Effects of the dietary protein level on the microbial composition and metabolomic profile in the hindgut of the pig. Anaerobe. 38:61–69. doi:10.1016/j.anaerobe.2015.12.009.

Zimmerman J, Benfi eld D, Murtaugh M, Osorio F, Stevenson G, Torremorell M. 2006. Porcine reproductive and respiratory syndrome virus. In: Diseases of Swine (9th edition). Straw BE, D'Allaire S, Zimmerman J, Taylor DJ (eds). Blackwell Publishing Company, Ames Iowa; p. 387–418

Zimmermann, J. A., M. L. Fusari, E. Rossler, J. E. Blajman, A. Romero-Scharpen, D. M. Astesana, C. R. Olivero, A. P. Berisvil, M. L. Signorini, M. V. Zbrun, L. S. Frizzo, and L. P. Soto. 2016. Effects of probiotics in swines growth performance: A meta-analysis of randomised controlled trials. Anim. Feed Sci. Technol. 219:280–293. doi:10.1016/j.anifeedsci.2016.06.021. Available from: http://dx.doi.org/10.1016/j.anifeedsci.2016.06.021

CHAPTER 2.   THE EFFECT OF INDIVIDUAL MCFA ON PORCINE REPRODUCTIVE RESPIRATORY SYNDROME VIRUS REPLICATION IN MARC-145 CELLS

2.1   Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is estimated to cost the US Swine Industry $664 million annually in production losses. Therefore, the objective of this experiment was to evaluate the effect of MCFA on PRRSV replication in vitro using MARC-145 cells, which are a subclone of the African Green Monkey Kidney MA104 cells, that are highly permissive to PRRSV. Individual MCFA (C6: caproic acid, C8: caprylic acid, C10: capric acid, and C12: lauric acid) were used in in vitro analysis to determine the impact on viral replication of PRRSV. Each MCFA was evaluated at 7 concentrations ranging from 1 to 1000 µg/mL with Type II North American (P-129) and Type I European (Lelystad) PRRSV. Viral replication was determined using FITC labeled IgG anti-PRRSV monoclonal antibody (SDOW-17-F RTI Labs Brookings South Dakota) and median tissue culture infectious dose Log_{10} $TCID_{50}$/mL values were calculated. Data were analyzed using the Proc Mixed procedure of SAS. Adding caproic acid (C6) had no effect on viral replication of PRRSV in MARC-145 cells. Caprylic acid (C8) reduced ($P < 0.01$) viral replication of PRRSV at the highest concentration of 1000 µg/mL in type II and I virus strains. Capric acid (C10) reduced ($P < 0.01$) viral replication at 300 µg/mL with type II virus strain and reduced ($P < 0.01$) viral replication of PRRSV at concentrations of 300 and 1000 µg/mL with type I virus strain. Lauric acid (C12) reduced ($P < 0.01$) viral replication of PRRSV at concentrations of 100 and 300 µg/mL with type II and I virus strains. In conclusion, the results of this experiment demonstrate that caprylic, capric and lauric acids were able to reduce PRRSV replication in MARC-145 cells with a 1.2 to 4.2 log reduction.

2.2   Introduction

The swine industry has been impacted by PRRSV for over 30 years. PRRSV is an enveloped single, positive strand RNA virus in the Arteriviridae family of viruses in the order of Nidovirales. PRRSV infection results in reproductive failure in sows and gilts as well as respiratory illness in all phases of pig production; resulting in an estimated $664 million in annual production losses for the US Swine Industry (Holtkamp et al., 2013). Veterinarians and producers have used several PRRSV prevention strategies over the years including reduced animal movement and mixing, heightened biosecurity measures, and herd vaccination. Control or prevention of PRRSV has been challenging due to persistently infected animals in the herd, high transmission rates between animals, and rapid development of genetic variants. Additional challenges include genetic evolution of highly virulent strains identified as recently as 2020, which increase the genetic diversity of PRRSV in global pig producing regions (Jiang et al., 2020; Kvisgaard et al., 2020; Park et al., 2020). This robust genetic diversity in PRRSV has caused a lack of vaccine efficacy and cross protection between strains (Butler et al., 2014).

The primary target of PRRSV is porcine alveolar macrophages. However, the MARC-145 cell line, a subclone of the African Green Monkey Kidney cell line, are susceptible to PRRSV infection and have been used in in vitro testing (Kim et al., 1993). The mechanisms involved in PRRSV attachment and entry into the cell use glycoproteins (GP3, GP4, GP5, and M) on the viral envelope that bind to cellular receptors CD163, heparan sulfate, and sialodhesin (Delputte et al., 2002 and 2007; Guo et al., 2017). Following endocytosis and membrane fusion, the viral genome is released into the cytoplasm, leading to viral replication. Prevention of PRRSV infection could

be achieved by inhibiting attachment and entry into the macrophages or the release of the viral genome in the cytoplasm.

MCFA have been used in swine industry diets to promote animal performance and as alternatives to antibiotics (Gebhardt et al., 2020; Thomas et al., 2020). MCFA ranging from C6 to C12 and have been reported to be effective against a wide variety of pathogens (Yoon et al., 2018) and pose a reduced risk of bacterial resistance. The primary proposed mode of action of MCFA is disruption of bacterial membranes and virus envelopes (Kim and Rhee 2013; Jackman et al., 2020). MCFA have immunomodulatory effects in the animal, specifically with T cell lymphocytes, leading to a TH1 and TH17 response through the GPR84 receptor (Huang et al., 2014). Additionally MCFA have been reported to be anti-inflammatory with reduced cytokine production (Zhang et al., 2016) and improved immune response (Martínez-Vallespín et al., 2016). Understanding that PRRSV infection leads to an increased inflammation in lung tissue and is immune suppressive, provides a basis for investigating MCFA supplementation in a PRRSV challenge.

2.3   Materials and Methods

2.3.1   General

This was the first in a series of experiments to test the hypothesis that MCFA could inhibit or reduce PRRSV infection and working towards our overall hypothesis that providing MCFA to pigs through feed could inhibit or reduce PRRSV infection. This experiment was designed to test individual MCFA (C6, C8, C10, and C12) on their effectiveness to reduce PRRSV replication in MARC-145 cells. Type II and Type I PRRSV strains were used to be an indicator of heterologous protection and determine if individual MCFA had different effects by strain. The MCFA were

tested at varying concentrations ranging from 1 to 1000 µg/mL. The PRRSV was mixed in a 50:50 mixture with MCFA concentrations or growth media prior to inoculation of MARC-145 cells. All tests were conducted in triplicate with a plate to plate control as a quality control measure to monitor viral contamination.

Data were analyzed using the PROC MIXED procedure in SAS 9.4 (SAS Institute INC., Cary, NC), with plate as the experimental unit. Median tissue culture infectious dose Log_{10} $TCID_{50}$/mL is defined as the dilution of virus required to infect 50% of the cell monolayer. Log_{10} $TCID_{50}$/mL values were calculated (Δt = the number of inoculated wells at *t and below divided by the number of wells inoculated at each dilution -0.5; *t = the lowest virus dilution demonstrating 100% infectivity; [for example when *t = viral dilution 4; 10 wells inoculated at each virus dilution; and 2 wells below dilution 4 are positive; $Log_{10}TCID_{50}$/ml= LOG10(10^(4+((10+2)/10)-0.5)/0.5]) based on the Karber method for each treatment (Karber, 1931). Data are presented as LS means with mean separation determined using the Pdiff statement if the main treatment effect was significant ($P < 0.05$). Statistical significance was defined as $P \leq 0.05$ and a tendency was defined as $0.05 < P \leq 0.10$.

2.3.2   Cell Culture

Meat Animal Research Center -145 (MARC-145) cells, which are a subclone of African Green Monkey Kidney MA104 cells, that are highly permissive to PRRSV, were used as an immortal cell model. MARC-145 cells were cultured in growth media consisting of Dulbecco's Modified Eagles Medium (DMEM, Science Cell, #09221) supplemented with 10% heat inactivated fetal bovine serum (Sigma, #F4135), 50 µL/500 mL of gentamicin, 5mL/500 mL of antibiotic/antimycotic solution (100x) and 5mL/500 mL of L-glutamine (200nM). Cells were maintained in 250 mL/75 cm^2 tissue culture flasks in a 37 ºC incubator with 5% CO_2. Cells were

trypsinized with trypsin/EDTA solution (Science Cell # 0103) and seeded in 96 well flat bottom tissue culture plates with low evaporation lid (VWR, #0132). Type II North American (P129) and Type I European (Lelystad) PRRSV Strains were propagated in MARC-145 cells to prepare virus stocks and stored at -80ºC in 1mL aliquots.

2.3.3 Virus Infection

MARC-145 cells were seeded onto 96 well tissue culture plates and incubated at 37 ºC in a 5% CO_2 environment for 48 h. MCFA concentrations were prepared through serial dilutions in growth media to achieve 1, 3, 10, 30, 100, 300, and 1000 µg/mL concentrations. Virus dilutions were prepared via 8 serial dilutions to achieve ($1x10^{-1}$ to $1x10^{-8}$ viral infective particles/mL) virus concentrations. Virus concentrations were then used to make a 50:50 mixture of virus and MCFA or growth media to be used for inoculation. After the 48 h incubation, wells were washed with growth media and then exposed to MCFA concentrations at a volume of 100 µL/well. Plates were then incubated for 2 h at 37 ºC in a 5% CO_2 environment. Immediately following the 2 h incubation, wells were washed with growth media and inoculated with 100 µL of the 50:50 mixture of virus and MCFA or growth media. Plates were then incubated for 1 h at 37 ºC in a 5% CO_2 environment and then virus was removed, and plates were washed with growth media. MCFA concentrations were added to each well at a volume of 100 µL/well, and plates were incubated for 48 h at 37 ºC in a 5% CO_2 environment.

2.3.4 Immunofluorescence Staining

MARC-145 cell plates were removed from the incubator after 48 h and wells were washed with 100 µL of phosphate buffered saline (PBS, Rockland Inc., #MB-008). Wells were fixed with 50 µL of 80% acetone and plates were incubated at room temperature for 10 minutes. Acetone was

removed, and plates were dried for 4h at room temperature. Wells were washed with 100 µL of PBS, blocked with 100 µL of bovine serum albumin blocking buffer (BSA, Jackson Immuno Research Lab, Inc., #001-000-162) and incubated at room temperature for 30 minutes. Wells were washed with 100 µL of PBS, stained with 50 µL of FITC labeled IgG anti-PRRSV monoclonal antibody (SDOW17-F RTI Labs Brookings, South Dakota) and incubated at 37 ºC in a 5% CO_2 environment for 30 minutes. Stained wells were washed twice with 100 µL of PBS and visualized under a UV microscope to determine viral replication within the MARC-145 cells. The median tissue culture infective dose Log_{10} $TCID_{50}$/mL was calculated for each MCFA concentration and control (Karber, 1931).

2.4   Results of In vitro PRRSV Replication in MARC-145

Incubation of MARC-145 cells with caproic acid (C6) at concentrations of 1-1000 µg/mL prior to and after inoculation with Type II North American (P129) (Figure 2.1) or Type I European (Lelystad) PRRSV (Figure 2.2) did not alter viral replication ($P > 0.10$). However, incubation of MARC-145 cells with caprylic (C8), capric (C10), and lauric (C12) acid prior to and after inoculation with Type II and Type I PRRSV did lead to reductions in viral replication. In general, the effective dose required to result in a reduction in Log_{10} $TCID_{50}$/mL ($P < 0.05$) viral replication decreased as chain length increased.

Specifically, caprylic acid (C8) resulted in 2.02 and 3.02 log reductions in Log_{10} $TCID_{50}$/mL ($P < 0.01$) viral replication of the Type II North American (P129, Figure 2.3) and Type I European (Lelystad, Figure 2.4) PRRSV, respectively, when supplied at 1000 µg/mL. Lower concentrations of caprylic acid were unable to reduce viral replication (P > 0.05).

Reductions in viral replication, as determined by $Log_{10}TCID_{50}$/mL, were observed at 300 µg/mL of capric acid (C10) for Type II (3.23 log reduction; Figure 2.5; $P < 0.01$) and Type I (3.85

log reduction; Figure 2.6; $P < 0.01$) PRRSV. Increasing concentrations of capric acid to 1000 µg/mL resulted in a further reduction in Log_{10} $TCID_{50}$/mL (4.17 log reduction; $P < 0.01$) for Type I European (Lelystad) PRRSV. However, 1000 µg/mL of capric acid in conjunction with Type II North American (P129) PRRSV resulted in the removal of the MARC-145 cells from the microtiter plate, making it impossible to evaluate the effectiveness of higher doses in vitro.

Lauric acid (C12) resulted in 0.90 and 1.2 log reductions in Log_{10} $TCID_{50}$mL ($P < 0.01$) viral replication of Type II North American (P129; Figure 2.7) and Type I European (Lelystad; Figure 2.8) PRRSV, respectively, when supplied at 100 µg/mL. Further reduction in Log_{10} $TCID_{50}$/mL resulted with increasing the concentration of lauric acid to 300 µg/mL (2.23 and 2.63 log reductions; $P < 0.01$) for Type II and Type I, respectively. Lauric acid in combination with both PRRSV strains at 1000 µg/mL resulted in the MARC-145 cells being removed from the microtiter plate, making it impossible to determine the effectiveness of higher doses in vitro.

2.5 Discussion

The objective of this experiment was to determine the effect of individual MCFA on PRRSV replication in two genetically different virus strains using MARC-145 cells. The MCFA used were C6, C8, C10, and C12 as previous research had reported antibacterial properties (Kabara et al., 1977) of these MCFA. More recent studies have reported MCFA have efficacy against envelope viruses (Thormar et al., 1987; Jackman et al., 2020b) as well as bacteria (Yoon et al., 2018; Kovanda et al., 2019). Our results indicate that C8, C10, and C12 are effective at reducing PRRSV replication in MARC-145 cells. There are differences in the effectiveness between MCFA as it appears to be dependent on concentration and chain length. Our data suggests that increasing chain length decreases the effective concentration as C12 is effective at reducing viral replication at 100 µg/mL in both virus strains while C10 is effective at 300 µg/mL and C8 is effective at 1000

µg/mL. The varying degree of effectiveness with MCFA in our experiment is in agreement with previous research (Lerner et al., 2020) that reported the effectiveness at reducing viral particles in feed is also increased with chain length at lower concentrations when comparing C5, C6, C8, and C10. The antibacterial properties of MCFA are also dependent upon chain length with shorter chains being more effective against gram-negative bacteria and longer chains being more effective against gram-positive bacteria (Gebhardt et al., 2020).

Feed mitigation is an area of increased research focus since the emergence of PEDV in the US Swine Industry in 2013. The use of MCFA to reduce viral transmission of PEDV via feed has been investigated using individual MCFA as well as a combination of MCFA. The results reported higher cycle threshold (Ct) values with MCFA present in the feed and the inhibition of PEDV infection in an in vivo model (Lerner et al., 2020). PRRSV and PEDV are two viruses of high economic importance to the US Swine Industry and both are enveloped RNA viruses. Recent research has shown MCFA to be effective against both viruses in feed (Cochrane et al., 2016; Dee et al., 2020; Lerner et al., 2020). PRRSV is mainly thought of as aerosol transmission threat, and not transmitted through feed or feed ingredients. However, PRRSV has been detected in feed samples and present in oral fluids and serum samples of pigs consuming positive feed (Dee et al., 2020). MCFA added in feed prior to inoculation with PRRSV and PEDV have inhibited viral transmission of PRRSV and PEDV in an in vivo model (Dee et al., 2020).

This experiment focused on the impact of individual MCFA and their antiviral activity against PRRSV in cell culture. The results of this experiment support our hypothesis that MCFA can reduce viral replication of PRRSV in MARC-145 cells. One effect noted on the higher concentrations of longer chain MCFA C10 and C12 was the removal of cells from the microtiter plates. This should be considered in future research to fully evaluate the effectiveness of MCFA

at higher concentrations. The efficacy at lower concentrations when using combinations of MCFA have been reported in feed mitigation research (Lerner et al., 2020). C10 and C12 having efficacy at 300 and 100 µg/mL, leads to the approach of using a smaller step up program from 100 to 500 µg/mL for each MCFA combination. In conclusion, C8, C10, and C12 should be investigated in combination with each other to evaluate if any synergies exist to reduce PRRSV replication. Next steps would be to conduct additional research on the mode of action through which MCFA reduce viral replication of PRRSV and work towards a nutritional application of MCFA for swine.

2.6   Literature Cited

Butler, J. E., K. M. Lager, W. Golde, K. S. Faaberg, M. Sinkora, C. Loving, and Y. I. Zhang. 2014. Porcine reproductive and respiratory syndrome (PRRS): an immune dysregulatory pandemic. Immunol. Res. 59:81–108. doi:10.1007/s12026-014-8549-5. Available from: http://link.springer.com/10.1007/s12026-014-8549-5

Cochrane, R. A., A. R. Huss, G. C. Aldrich, C. R. Stark, and C. K. Jones. 2016. Evaluating Chemical Mitigation of Salmonella Typhimurium ATCC 14028 in Animal Feed Ingredients. J. Food Prot. 79:672–676. doi:10.4315/0362-028X.JFP-15-320. Available from: https://meridian.allenpress.com/jfp/article/79/4/672/174449/Evaluating-Chemical-Mitigation-of-Salmonella

Dee, S. A., M. C. Niederwerder, G. Patterson, R. Cochrane, C. Jones, D. Diel, E. Brockhoff, E. Nelson, G. Spronk, and P. Sundberg. 2020. The risk of viral transmission in feed: What do we know, what do we do? Transbound. Emerg. Dis. 67:2365–2371. doi:10.1111/tbed.13606. Available from: https://onlinelibrary.wiley.com/doi/10.1111/tbed.13606

Delputte, P. L., W. Van Breedam, I. Delrue, C. Oetke, P. R. Crocker, and H. J. Nauwynck. 2007. Porcine Arterivirus Attachment to the Macrophage-Specific Receptor Sialoadhesin Is Dependent on the Sialic Acid-Binding Activity of the N-Terminal Immunoglobulin Domain of Sialoadhesin. J. Virol. 81:9546–9550. doi:10.1128/JVI.00569-07. Available from: https://jvi.asm.org/content/81/17/9546

Delputte, P. L., N. Vanderheijden, H. J. Nauwynck, and M. B. Pensaert. 2002. Involvement of the Matrix Protein in Attachment of Porcine Reproductive and Respiratory Syndrome Virus to a Heparinlike Receptor on Porcine Alveolar Macrophages. J. Virol. 76:4312–4320. doi:10.1128/JVI.76.9.4312-4320.2002. Available from: http://jvi.asm.org/

Gebhardt, J. T., K. A. Thomson, J. C. Woodworth, S. S. Dritz, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, C. K. Jones, R. A. Cochrane, M. C. Niederwerder, S. Fernando, W. Abbas, and T. E. Burkey. 2020. Effect of dietary medium-chain fatty acids on nursery pig growth performance, fecal microbial composition, and mitigation properties against porcine epidemic diarrhea virus following storage. J. Anim. Sci. 98. doi:10.1093/jas/skz358. Available from: https://academic.oup.com/jas/article/doi/10.1093/jas/skz358/5638934

Guo, C., Z. Zhu, Y. Guo, X. Wang, P. Yu, S. Xiao, Y. Chen, Y. Cao, and X. Liu. 2017. Heparanase Upregulation Contributes to Porcine Reproductive and Respiratory Syndrome Virus Release. J. K. Pfeiffer, editor. J. Virol. 91. doi:10.1128/JVI.00625-17. Available from: https://jvi.asm.org/lookup/doi/10.1128/JVI.00625-17

Holtkamp, D. J., J. B. Kliebenstein, E. J. Neumann, J. J. Zimmerman, H. F. Rotto, T. K. Yoder, C. Wang, P. E. Yeske, C. L. Mowrer, and C. A. Haley. 2013. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. Available from: http://www.aasv.org/shap.html.

Huang, Q., D. Feng, K. Liu, P. Wang, H. Xiao, Y. Wang, S. Zhang, and Z. Liu. 2014. A medium-chain fatty acid receptor Gpr84 in zebrafish: Expression pattern and roles in immune regulation. Dev. Comp. Immunol. 45:252–258. doi:10.1016/j.dci.2014.03.017. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0145305X14000858

Jackman, J. A., R. D. Boyd, and C. C. Elrod. 2020a. Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation. J. Anim. Sci. Biotechnol. 11:44. doi:10.1186/s40104-020-00446-1. Available from: https://jasbsci.biomedcentral.com/articles/10.1186/s40104-020-00446-1

Jackman, J. A., A. Hakobyan, H. Zakaryan, and C. C. Elrod. 2020b. Inhibition of African swine fever virus in liquid and feed by medium-chain fatty acids and glycerol monolaurate. J. Anim. Sci. Biotechnol. 11:114. doi:10.1186/s40104-020-00517-3. Available from: https://jasbsci.biomedcentral.com/articles/10.1186/s40104-020-00517-3

Jiang, Y., G. Li, L. Yu, L. Li, Y. Zhang, Y. Zhou, W. Tong, C. Liu, F. Gao, and G. Tong. 2020. Genetic Diversity of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) From 1996 to 2017 in China. Front. Microbiol. 11. doi:10.3389/fmicb.2020.00618. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2020.00618/full

Kabara, J. J., R. Vrable, and M. S. F. Lie Ken Jie. 1977. Antimicrobial lipids: Natural and synthetic fatty acids and monoglycerides. Lipids. 12:753–759. doi:10.1007/BF02570908. Available from: http://doi.wiley.com/10.1007/BF02570908

Kärber, G. 1931. Beitrag zur kollektiven Behandlung pharmakologischer. Beitrag zur Kollekt. Behandlung pharmakologischer Reihenversuche. 1–4.

Kim, H. S., J. Kwang, I. J. Yoon, H. S. Joo, and M. L. Frey. 1993. Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line Brief Report. Springer-Verlag.

Kim, S. A., and M. S. Rhee. 2013. Marked synergistic bactericidal effects and mode of action of medium-chain fatty acids in combination with organic acids against Escherichia coli O157: H7. Appl. Environ. Microbiol. 79:6552–6560. doi:10.1128/AEM.02164-13.

Kovanda, L., W. Zhang, X. Wei, J. Luo, X. Wu, E. R. Atwill, S. Vaessen, X. Li, and Y. Liu. 2019. In Vitro Antimicrobial Activities of Organic Acids and Their Derivatives on Several Species of Gram-Negative and Gram-Positive Bacteria. Molecules. 24:3770. doi:10.3390/molecules24203770. Available from: https://www.mdpi.com/1420-3049/24/20/3770

Kvisgaard, L. K., C. S. Kristensen, P. Ryt-Hansen, K. Pedersen, T. Stadejek, R. Trebbien, L. O. Andresen, and L. E. Larsen. 2020. A recombination between two Type 1 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV-1) vaccine strains has caused severe outbreaks in Danish pigs. Transbound. Emerg. Dis. 67:1786–1796. doi:10.1111/tbed.13555. Available from: https://onlinelibrary.wiley.com/doi/10.1111/tbed.13555

Lerner, A. B., R. A. Cochrane, J. T. Gebhardt, S. S. Dritz, C. K. Jones, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. Bai, E. Porter, J. Anderson, P. C. Gauger, D. R. Magstadt, J. Zhang, B. Bass, T. Karnezos, B. de Rodas, and J. C. Woodworth. 2020. Effects of medium chain fatty acids as a mitigation or prevention strategy against porcine epidemic diarrhea virus in swine feed. J. Anim. Sci. 98. doi:10.1093/jas/skaa159. Available from: https://academic.oup.com/jas/article/doi/10.1093/jas/skaa159/5843591

Martínez-Vallespín, B., W. Vahjen, and J. Zentek. 2016. Effects of medium-chain fatty acids on the structure and immune response of IPEC-J2 cells. Cytotechnology. 68:1925–1936. doi:10.1007/s10616-016-0003-1. Available from: http://link.springer.com/10.1007/s10616-016-0003-1

Park, C., J. H. Baek, S. H. Cho, J. Jeong, C. Chae, S.-H. You, and S.-H. Cha. 2020. Field porcine reproductive and respiratory syndrome viruses (PRRSV) attenuated by codon pair deoptimization (CPD) in NSP1 protected pigs from heterologous challenge. Virology. 540:172–183. doi:10.1016/j.virol.2019.10.019. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0042682219303083

Thomas, L. L., J. C. Woodworth, M. D. Tokach, S. S. Dritz, J. M. DeRouchey, R. D. Goodband, H. E. Williams, A. R. Hartman, D. J. Mellick, D. M. McKilligan, and A. M. Jones. 2020. Evaluation of different blends of medium-chain fatty acids, lactic acid, and monolaurin on nursery pig growth performance12. Transl. Anim. Sci. 4:548–557. doi:10.1093/tas/txaa024.

Thormar, H., C. E. Isaacs, H. R. Brown, M. R. Barshatzky, and T. Pessolano. 1987. Inactivation of enveloped viruses and killing of cells by fatty acids and monoglycerides. Antimicrob. Agents Chemother. 31:27–31. doi:10.1128/AAC.31.1.27. Available from: http://aac.asm.org/cgi/doi/10.1128/AAC.31.1.27

Yoon, B., J. Jackman, E. Valle-González, and N.-J. Cho. 2018. Antibacterial Free Fatty Acids and Monoglycerides: Biological Activities, Experimental Testing, and Therapeutic Applications. Int. J. Mol. Sci. 19:1114. doi:10.3390/ijms19041114. Available from: http://www.mdpi.com/1422-0067/19/4/1114

Zhang, M. S., A. Sandouk, and J. C. D. Houtman. 2016. Glycerol Monolaurate (GML) inhibits human T cell signaling and function by disrupting lipid dynamics. Sci. Rep. 6:30225. doi:10.1038/srep30225. Available from: http://www.nature.com/articles/srep30225

Figure 2.1 Incubation of M145 cells with caproic acid (C6) prior to and after inoculation with Type II North American (P129) PRRSV did not ($P > 0.05$) affect PRRSV replication. Log10 $TCID_{50}$/mL values were calculated based on the Karber method.
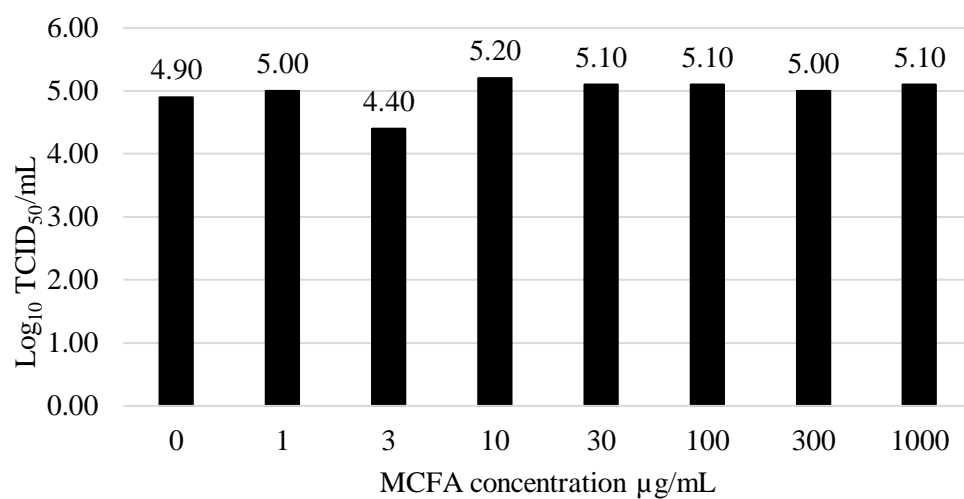
Figure 2.2 Incubation of M145 cells with caproic acid (C6) prior to and after inoculation with Type I European (Lelystad) PRRSV did not ($P > 0.05$) affect PRRSV replication. Log_{10} $TCID_{50}$/mL values were calculated based on the Karber method.
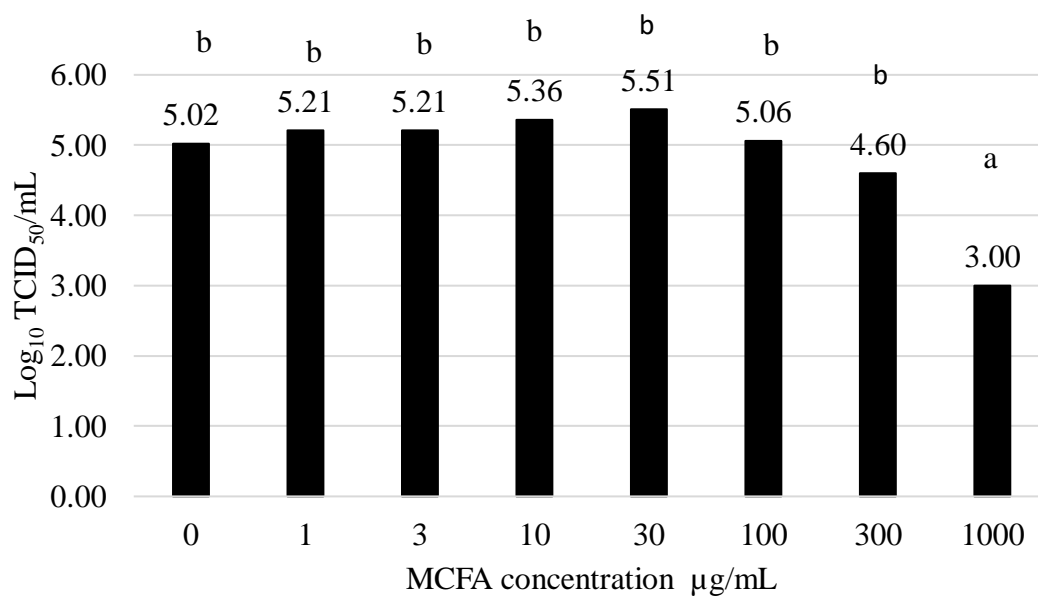
Figure 2.3 Incubation of M145 cells with caprylic acid (C8) prior to and after inoculation with Type II North American (P129) PRRSV resulted in a 2.02 log reduction ($P < 0.01$) in PRRSV replication at the 1000 µg/mL concentration. Log_{10} $TCID_{50}$/mL values were calculated based on the Karber method. [ab]Letters indicated differences ($P < 0.05$).
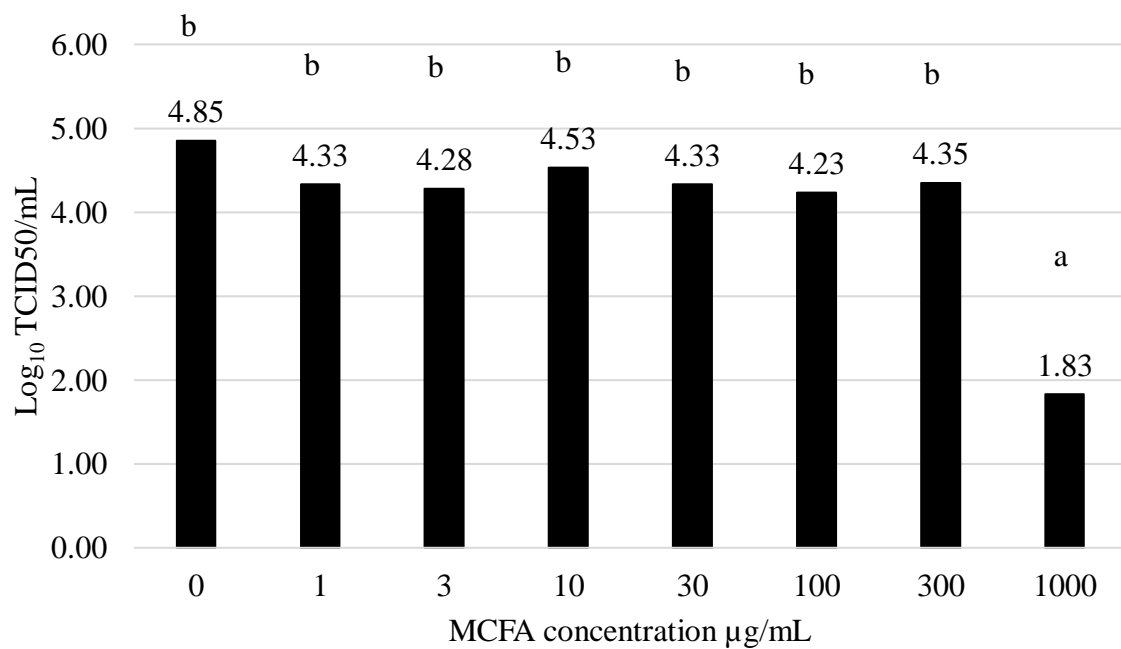
Figure 2.4 Incubation of M145 cells with caprylic acid (C8) prior to and after inoculation with Type I European (Lelystad) PRRSV resulted in a 3.02 log reduction ($P < 0.01$) in PRRSV replication at the 1000 µg/mL concentration. Log_{10} $TCID_{50}$/ml values were calculated based on the Karber method. [ab]Letters indicated differences ($P < 0.05$).
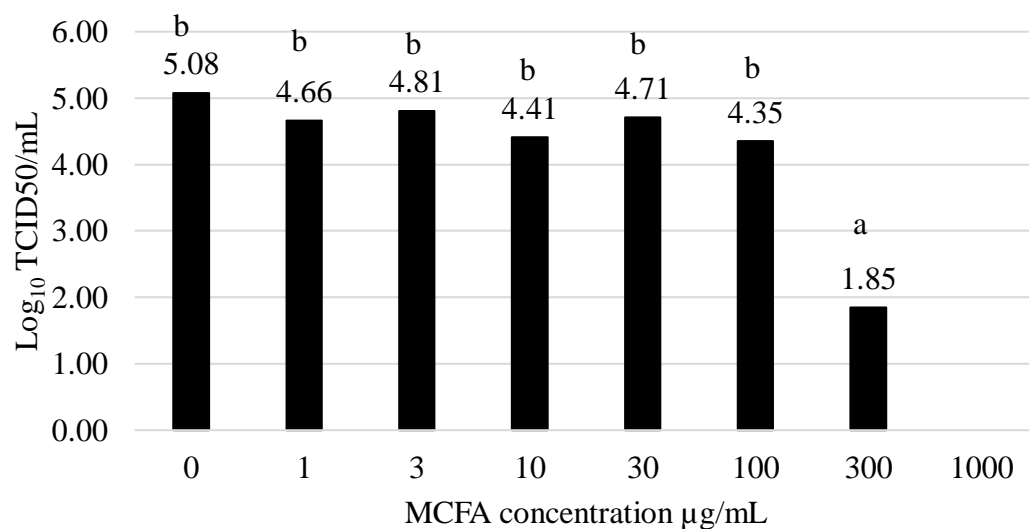
Figure 2.5 Incubation of M145 cells with capric acid (C10) prior to and after inoculation with Type II North American (P129) PRRSV resulted in a 3.23 log reduction ($P < 0.01$) in PRRSV replication at the 1000 µg/mL concentration. Log_{10} $TCID_{50}$/ml values were calculated based on the Karber method. [ab]Letters indicated differences ($P < 0.05$).
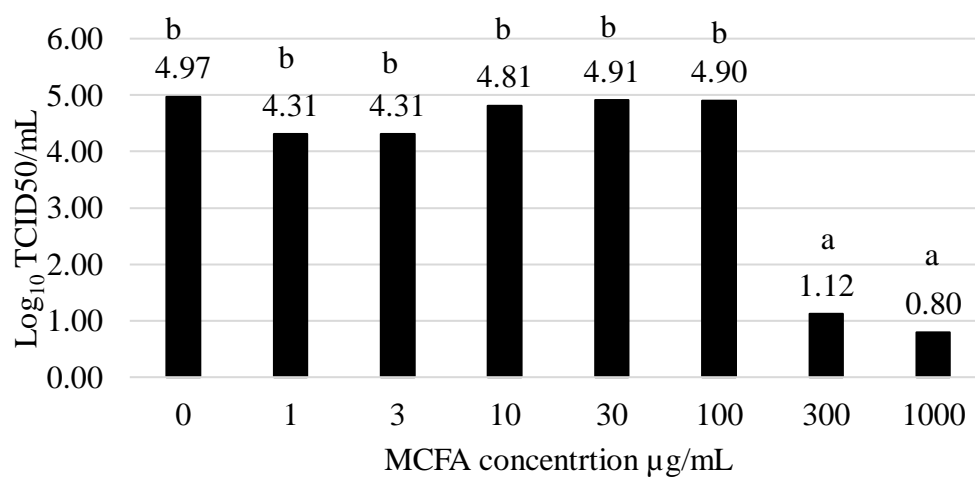
Figure 2.6 Incubation of M145 cells with capric acid (C10) prior to and after inoculation with Type I European (Lelystad) PRRSV resulted in a 3.85 and 4.17 log reduction ($P < 0.01$) in PRRSV replication at the 300 µg/mL and 1000 µg/mL concentration respectively. Log_{10} $TCID_{50}$/ml values were calculated based on the Karber method. [ab]Letters indicated differences ($P < 0.05$).
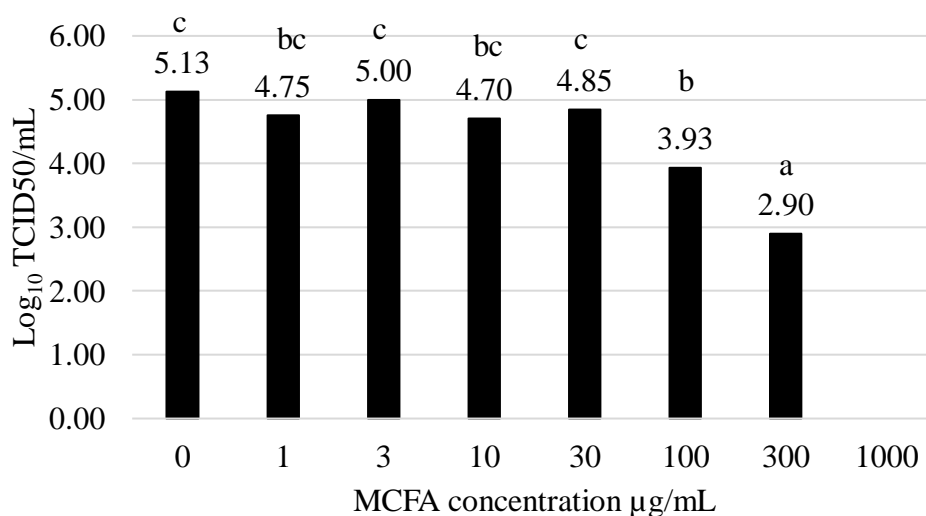
Figure 2.7 Incubation of M145 cells with lauric acid (C12) prior to and after inoculation with Type II North American (P129) PRRSV resulted in a 1.2 and 2.23 log reduction ($P < 0.01$) in PRRSV replication at the 100 µg/mL and 300 µg/mL concentration, respectively. Log10 $TCID_{50}$/mL values were calculated based on the Karber method. [abc]Letters indicated differences ($P < 0.05$).
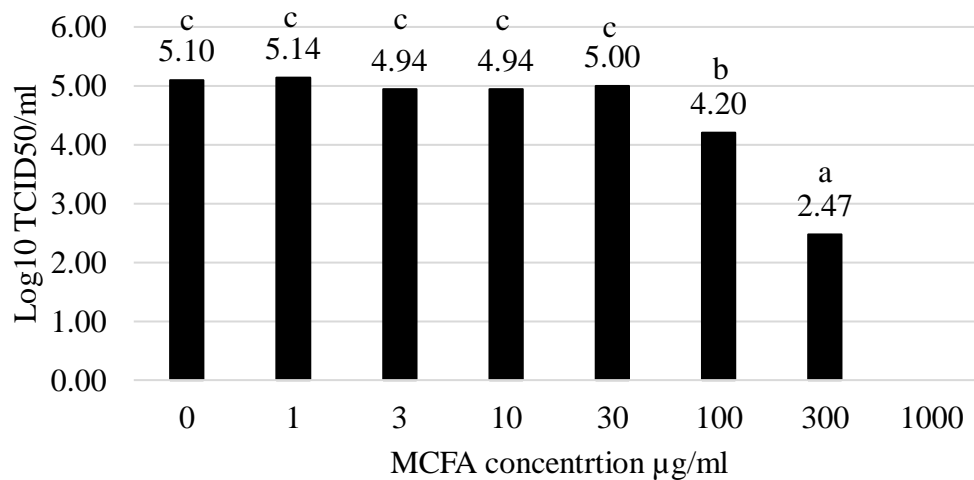
Figure 2.8 Incubation of M145 cells with lauric acid (C12) prior to and after inoculation with Type I European (Lelystad) PRRSV resulted in a 0.9 and 2.63 log reduction (P < 0.01) in PRRSV replication at the 100 µg/mL and 300 µg/mL concentration respectively. Log_{10} $TCID_{50}$/mL values were calculated based on the Karber method. [abc] Letters indicated differences (*P* < 0.05).

CHAPTER 3.   THE EFFECT OF MCFA COMBINATIONS ON PORCINE REPRODUCTIVE RESPIRATORY SYNDROME VIRUS REPLICATION IN MARC-145 CELLS

3.1   Abstract

Porcine reproduction and respiratory syndrome virus (PPRSV) prevention strategies include decreased animal movement, heightened biosecurity, and vaccination. However, even with these strategies 20-30% of US swine herds experience PRRSV infections each year. Therefore, the objective of this experiment was to evaluate the effect of medium chain fatty acids (MCFA) on PRRSV replication in MARC-145 cells. Individual MCFA that were found to be effective in our previous research (Chapter 2; C8 caprylic acid, C10 capric acid, and C12 lauric acid) were used in several combinations to determine the impact on replication of PRRSV in MARC-145 cells. Each MCFA combination (C8:C10, C8:C12, C10:C12, and C8:C10:C12) was mixed in a 1:1 ratio (wt:wt) and evaluated at 6 concentrations ranging from 50 to 500 µg/mL of growth media. Type II North American (P-129) and Type I European (Lelystad) PRRSV were evaluated. Viral replication was determined using FITC labeled IgG anti-PRRSV monoclonal antibody (SDOW-17-F RTI Labs Brookings South Dakota) and median tissue culture infectious dose Log_{10} $TCID_{50}$/mL values were calculated. Data were analyzed using the Proc Mixed procedure of SAS. C8:C10, C8:C12, C10:C12, and C8:C10:C12 combinations all reduced ($P < 0.01$) viral replication of PRRSV at concentrations of 200-500 µg/mL in both type II and type I PRRSV strains. The MCFA combination C8:C10:C12 was used to determine if the reduction in viral replication was the result of treating the cells or the virus. Incubating only the MARC-145 cells with C8:C10:C12 but not the virus alone resulted in a reduction ($P < 0.01$) in viral replication with concentrations of 200-500 µg/mL in both PRRSV strains. In conclusion, results of this experiment demonstrated that

MCFA combinations used were able to reduce PRRSV viral replication in MARC-145 cells and the effect of MCFA was the result of an effect on the MARC-145 cells, not the PRRSV.

Keywords: MCFA, PRRSV, Viral Replication, Swine

3.2 Introduction

PRRSV is a challenging pathogen for swine producers, veterinarians, and nutritionists to completely understand and prevent. PRRSV is an aerosol pathogen and therefore can travel many miles, spreading the disease to neighboring barns. PRRSV is an enveloped, single positive stranded RNA virus in the Arteriviridae family of viruses in the order of Nidovirales. PRRSV is prone to mutations adding to the complexity of disease management and prevention. The genetic diversity and variant strains have prevented cross protective immunity from vaccines (Jiang et al., 2020). The ability of PRRSV to suppress immune function and evade the host immune response causes infected herds to experience recurring breaks and prolonged production losses. Prevention strategies implemented by veterinarians and producers are decreased animal movements, heightened biosecurity, and vaccine programs. Even with these strategies PRRSV infections are estimated to cost the US Swine Industry $664 million annually in production losses (Holtkamp et al., 2013). Jiang et al., (2020) reported that genetic variants and recombination have led to more virulent strains and greater genetic diversity, hindering PRRSV control and vaccination strategies.

MCFA have been reported to be effective against a wide variety of bacteria and viruses (Wang & Johnson, 1992; Lee, 2015; Du et al., 2017; Yoon et al., 2018; Jackman et al., 2020) making them viable candidates as alternative to antibiotics in animal production. MCFA target the membrane of pathogens and thereby reduce the opportunity to develop resistance (Desbois & Smith, 2010; Schlievert & Peterson, 2012).

Specific to PRRSV, MCFA are of interest due to their ability to disrupt the bilipid envelope (Du et al., 2017). There are three cellular receptors/mediators known in the pathway of PRRSV entry into macrophages (De Baere et al., 2012): 1) heparan sulphate is an important external attachment factor on the cell membrane, 2) porcine sialoadhesin (PoSn) influences binding and internalization of virus with the receptor, and 3) hemoglobin scavenger receptor CD163 is involved in membrane fusion and release of the viral genome (Van Breedam et al., 2010). PRRSV attachment and endocytosis into the cell uses glycoproteins (GP3, GP4, GP5, and M) on the viral envelope to bind to cellular receptors (Delputte et al., 2002 and 2007, Guo et al., 2017). Endocytosis of PRRSV leads to a critical step of membrane fusion that releases the viral genome into the cytoplasm leading to viral replication (Mas & Melero, 2013). Prevention of PRRSV infection could be achieved by inhibiting attachment to the cell or inhibition of membrane fusion. MCFA could interrupt PRRSV attachment to the cellular receptor through disruption of the viral envelope or membrane fusion and inhibit the release of the viral genome.

Therefore, our hypothesis was that combinations of MCFA based on previous data from individual MCFA (Chapter 2) would reduce viral replication of PRRSV at lower concentrations in MARC-145 cells infected with PRRSV. This research was designed to further our understanding in the application of MCFA to inhibit or reduce PRRSV replication in MARC-145 cells.

3.3   Materials and Methods

3.3.1   General

This was the second experiment in a series to test the hypothesis that MCFA could inhibit or reduce PRRSV infection and building towards our overall hypothesis that providing MCFA to pigs through feed would inhibit or reduce PRRSV infection. This experiment was designed to test

combinations of MCFA (C8:C10, C8:C12, C10:C12, and C8:C10:C12) on their effectiveness to reduce PRRSV replication in MARC-145 cells. Type II North American (P129) and Type I European (Lelystad) PRRSV strains were used as an indicator of heterologous protection and to determine if MCFA combinations had strain specific effects. The MCFA combinations were tested at concentrations ranging from 50 to 500 µg/mL of growth media. The PRRSV was mixed in a 50:50 mixture with the MCFA concentrations prior to inoculation of MARC-145 cells. All tests were conducted in triplicate with plate to plate controls as a quality control to monitor viral contamination.

Data were analyzed using the PROC MIXED procedure in SAS 9.4 (SAS Institute INC., Cary, NC), with plate as the experimental unit. Median tissue culture infectious dose Log_{10} $TCID_{50}$/mL is defined as the dilution of virus required to infect 50% of the cell monolayers. Log_{10} $TCID_{50}$/mL values were calculated (Δt = the number of inoculated wells at *t and below divided by the number of wells inoculated at each dilution -0.5; *t = the lowest virus dilution demonstrating 100% infectivity; [for example when *t = viral dilution 4; 10 wells inoculated at each virus dilution; and 2 wells below dilution 4 are positive; $Log_{10}TCID_{50}$/ml= LOG10(10^(4+((10+2)/10)-0.5)/0.5])based on the Karber method for each treatment (Karber, 1931). Data are presented as LS means with mean separation determined using the Pdiff statement if the main treatment effect was (P < 0.05). Statistical significance was defined as P ≤ 0.05 and a tendency was defined as 0.05 < P ≤ 0.10.

3.3.2 Cell Culture

Meat Animal Research Center -145 (MARC-145) cells, which are a subclone of African Green Monkey Kidney MA104 cells, that are highly permissive to PRRSV, were used as an immortal cell model. MARC-145 cells were cultured in growth media consisting of Dulbecco's

Modified Eagles Medium (DMEM, Science Cell, #09221) supplemented with 10% heat inactivated fetal bovine serum (Sigma, #F4135), 50 µL/500 mL of gentamicin, 5 mL/500 mL of antibiotic/antimycotic solution (100x; Gibco #15240) and 5 mL/500 mL of L-glutamine (200nM). Cells were maintained in 250 mL/75 cm^2 tissue culture flasks in a 37 ºC incubator with 5% CO_2. Cells were trypsinized with trypsin/EDTA solution (Science Cell # 0103) and seeded in 96 well flat bottom tissue culture plates with low evaporation lid (VWR, #0132). Type II North American (P129) and Type I European (Lelystad) PRRSV strains were propagated in MARC-145 cells to prepare virus stocks and stored at -80ºC in 1mL aliquots.

3.3.3   Virus Infection

MARC-145 cells were seeded onto 96 well tissue culture plates and incubated at 37ºC in a 5% CO_2 environment for 48 h. MCFA combinations were mixed in 1:1 ratios (wt:wt) of C8:C10, C8:C12, C10:C12, or C8:C10:C12. MCFA concentrations were prepared through serial dilutions in growth media to achieve 50, 100, 200, 300, 400, and 500 µg/mL concentrations. Viral dilutions were prepared via 8 serial dilutions to achieve ($1x10^{-1}$ to $1x10^{-8}$ viral infective particles/mL) virus concentrations. Virus concentrations were then used to make a 50:50 mixture of virus and MCFA or growth media to be used for inoculation. After the 48 h incubation, wells were washed with growth media and exposed to MCFA concentrations at a volume of 100 µL/well. Plates were then incubated for 2 h at 37ºC in a 5% CO_2 environment. Immediately following the 2 h incubation, wells were washed with growth media and inoculated with 100 µL of the 50:50 mixture of virus and MCFA or growth media. Plates were then incubated for 1 h at 37ºC in a 5% CO_2 environment and then washed with growth media. MCFA concentrations were added back to each well at a volume of 100 µL/well, and plates were incubated for 48 h at 37ºC in a 5% CO_2 environment.

3.3.4 Mode of action evaluation

Additional testing was conducted to determine if the effect of MCFA were mediated through MARC-145 cells, PRRSV, or both. The virus infection described above tested the combination of treating both MARC-145 cells and PRRSV. Therefore, procedures from above were modified to treat the MARC -145 cells or the PRRSV independently. MARC-145 cells were seeded onto 96 well tissue culture plates and incubated at 37 ℃ in a 5% CO_2 environment for 48 h. The MCFA combination of C8:C10:C12 was prepared and concentrations were made through serial dilutions in growth media to achieve 50, 100, 200, 300, 400, and 500 µg/mL concentrations. Viral dilutions were prepared via serial dilutions in growth media to achieve ($1x10^{-1}$ to $1x10^{-8}$ viral infective particles/mL) virus concentrations. Virus concentrations were then used to make a 50:50 mixture of virus and MCFA or virus and growth media to be used for inoculation. Testing the effect on MARC-145 cells was through MARC-145 exposure to MCFA concentrations. Testing the effect on the PRRSV alone was through virus exposure to MCFA in a 50:50 mixture. After the 48 h incubation, wells were washed with growth media and exposed to MCFA concentrations at a volume of 100 µL/well. Plates were then incubated for 2 h at 37℃ in a 5% CO_2 environment. Immediately following the 2 h incubation, wells were washed with growth media and inoculated with 100 µL of the 50:50 mixture of virus and MCFA or virus and growth media. Plates were then incubated for 1 h at 37℃ in a 5% CO_2 environment and then washed with growth media. MCFA concentrations or growth media were added to each well at a volume of 100 µL/well, and plates were incubated for 48 h at 37 ℃ in a 5% CO_2 environment.

3.3.5 Immunofluorescence Staining

MARC-145 cell plates were removed from the incubator after 48 h and wells were washed with 100 µL of phosphate buffered saline (PBS, Rockland Inc., #MB-008). Wells were fixed with

50 µL of 80% acetone and plates were incubated at room temperature for 10 minutes. Acetone was removed, and plates were then dried for 4 h at room temperature. Wells were washed with 100 µL of PBS, then blocked with 100 µL of bovine serum albumin blocking buffer (BSA, Jackson Immuno Research Lab, Inc., #001-000-162) and incubated at room temperature for 30 minutes. Wells were then washed with 100 µL of PBS and then 50 µL of FITC labeled IgG anti-PRRSV monoclonal antibody (SDOW17-F RTI Labs Brookings, South Dakota) was added to each well and incubated at 37 ℃ in a 5% CO_2 environment for 30 minutes. This monoclonal antibody recognizes a highly conserved epitope on the nucleocapsid protein of PRRSV. Wells were then washed twice with 100 µL of PBS and visualized under a UV microscope to determine viral replication within the MARC-145 cells. The wells having adherence of the antibody were bright green in color and were recorded as PRRSV infected. The median tissue culture infective dose Log_{10} $TCID_{50}$/mL was calculated for each MCFA concentration and control (Karber, 1931).

3.4   Results of In vitro PRRSV Replication in MARC-145

Incubation of MARC-145 cells and virus with MCFA combinations prior to and after inoculation with Type II North American (P129) and Type I European (Lelystad) PRRSV led to a reduction in viral replication..

The caprylic and capric acid combination (C8:C10) resulted in a 2.20 and 3.30 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) viral replication of Type II North American (P129) PRRSV at 200 and 300 µg/mL concentrations, respectively (Figure 3.1). Increasing concentrations of the caprylic and capric acid combination to 400 and 500 µg/mL resulted in 4.07 and 4.03 log reductions in Log_{10} $TCID_{50}$/mL viral replication, respectively ($P < 0.01$). The combination of caprylic and capric acid performed similar in Type I European (Lelystad) PRRSV (Figure 3.2) resulting in a 1.90,

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2.64, 4.14, and 4.10 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) viral replication at 200, 300, 400, and 500 µg/mL concentrations, respectively.

Reductions in viral replication, as determined by Log_{10} $TCID_{50}$/mL, were observed at 200 µg/mL for the combination of caprylic and lauric acid (C8:C12) with Type II (3.80 log reduction; Figure 3.3; $P < 0.01$) and Type I (3.44 log reduction; Figure 3.4; $P < 0.01$) PRRSV. Increasing the concentration of caprylic and lauric acid (C8:C12) to 300 µg/mL resulted in a 4.20 log reduction in Log_{10} $TCID_{50}$/mL viral replication with Type II ($P < 0.01$) and Type I ($P < 0.01$) PRRSV strains. Further increase in MCFA concentrations to 500 µg/mL did not further reduce viral replication.

Capric and lauric acid combination (C10:C12) resulted in a 2.14 and 2.94 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) viral replication of Type II North American (P129) PRRSV (Figure 3.5) at 200 and 300 µg/mL concentrations, respectively. Higher concentrations of capric and lauric acid resulted in a further 4.20 and 4.27 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) viral replication at 400 and 500 µg/mL, respectively. The combination of capric and lauric acid resulted in a 3.37, 4.20, 4.20, and 4.09 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) replication in Type I European (Lelystad) PRRSV at 200, 300, 400, and 500 µg/mL concentrations, respectively (Figure 3.6).

The caprylic, capric, and lauric acid combination (C8:C10:C12) resulted in a 2.97 and 2.54 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) viral replication for Type II North American (P129) (Figure 3.7) and Type I European (Lelystad) PRRSV (Figure 3.8), respectively, when supplied at 200 µg/mL. Higher concentrations further reduced viral replication in both PRRSV strains. Viral replication incurred a 4.20 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) at 300, 400, and 500 µg/mL for the Type II North American (P129) PRRSV. Viral replication of Type I European

(Lelystad) PRRSV was reduced ($P < 0.01$) by a 3.74, 3.77, and 3.77 log reduction in Log_{10} $TCID_{50}$/mL at 300, 400, and 500 µg/mL respectively.

In general, MCFA combinations reduced viral replication at concentrations of 200 µg/mL and above. To determine if the effect was on MARC-145 cells or PRRSV itself, each were independently incubated with MCFA. Type II North American (P129) (Figure 3.9) or Type I European (Lelystad) PRRSV (Figure 3.10) incubated with MCFA prior to inoculation did not affect viral replication ($P > 0.10$). However, incubation of MARC-145 cells with C8:C10:C12 prior to and after inoculation with Type II and Type I PRRSV did lead to a significant reduction in viral replication. Reductions in viral replication, as determined by Log_{10} $TCID_{50}$/mL, were observed at 200 µg/mL of C8:C10:C12 for Type II (1.34 log reduction; Figure 3.11; P <0.01) and Type I (1.56 log reduction; Figure 3.12; $P < 0.01$) PRRSV. Higher concentrations of C8:C10:12 led to 4.17, 4.27, and 4.27 log reduction in Log_{10} $TCID_{50}$/mL replication for Type II PRRSV at 300, 400, and 500 µg/mL concentrations, respectively ($P < 0.01$). C8:C10:C12 resulted in a 4.14 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) viral replication of the Type I PRRSV at concentrations of 300, 400, and 500 µg/mL. These results indicate that the inhibition observed with MCFA is a specific effect on MARC-145 cells and not the PRRSV itself.

3.5 Discussion

The objective of this study was to determine the effect of MCFA combinations on PRRSV replication in two genetically different PRRSV strains using MARC-145 cells. The MCFA combinations used were caprylic and capric acids (C8:C10), caprylic and lauric acids (C8:C12), capric and lauric acids (C10:C12), and caprylic, capric, and lauric acids (C8:C10:C12). These combinations were determined from previous research with individual MCFA (Chapter 2). The MCFA combinations were mixed in a 1:1 ratio on a wt:wt basis. Two genetically different strains

of PRRSV were used to gain an understanding of the potential anti-viral properties of MCFA between strains, because there is a lack of cross strain protection from current PRRSV vaccines. These results are aligned with the results from Chapter 2 and demonstrate a similar response of MCFA combinations both the Type II North American (P129) and Type I European (Lelystad) PRRSV strains. This indicates that MCFA could inhibit PRRSV replication and offer cross protection across different strains. Compared to the results reported in Chapter 2 the combination of MCFA appear to have synergistic responses as demonstrated with higher reduction in viral replication reported for all combinations. Our data is in agreement with earlier research that reported MCFA were effective in reducing viral infection in cell culture (Hilmarsson et al., 2005). Caprylic acid has been shown to be effective against enveloped viruses (Dichtelmuller et al., 2002).

Viruses are obligate intracellular parasites that utilize host cellular components in replication, assembly, and secretion (Heaton and Randall 2011; Mazzon and Mercer 2014). Envelope viruses modify lipid metabolism and alter intracellular membranes to enhance viral replication sites within the cell (Hsu et al., 2010). Fatty acids have been reported to modify cellular membrane structure that in turn can prevent membrane fusion (Van Meer et al., 2008; Lorizate et al., 2013), thereby inhibiting viral replication. Previous research on viral entry has reported cholesterol to be an important component of cell membranes and when cholesterol was depleted in the MARC-145 cells PRRSV infection was inhibited (Huang et al.,2011; Ying et al., 2011). Cholesterol through lipid rafts in the cellular membrane is critical to viral entry and fusion (Ono and Freed, 2005). Modifications to fatty acid metabolism have been reported to be inhibitory to PRRSV infection in vitro (Long et al., 2019). Our results indicate that MCFA reduce viral replication through an effect on MARC-145 cells and not by an effect on the virus prior to inoculation. These findings suggest that one possible mode of action of MCFA might be the modification of cell membrane

components and interference with the attachment or release of the virus resulting in reduced viral replication.

Multiple studies have reported the inhibition of pathogenic bacteria by MCFA (Kabara et al., 1977; Hanczakowska et al., 2010, 2011; Schlievert & Peterson, 2012; Yoon et al., 2018). MCFA support the growth of beneficial bacteria, through inhibition of pathogenic bacteria, enhancing digestion and nutrient utilization (Decuypere & Dierick, 2003; Calder, 2008; Baltić et al., 2017). MCFA can support gut function as a direct energy source for enterocytes, by promoting intestinal lining integrity (Zentek et al., 2011), and through anti-inflammatory actions (Zhang et al., 2016). Immune function is directly impacted by the immunomodulatory effects of MCFA via T cell lymphocyte cell signaling (Briscoe et al., 2003; Haghikia et al., 2015; Zhang et al., 2018) and improved immune responses (Martínez-Vallespín et al., 2016). Improved immune responses have a direct impact on animal performance due to the energy expensive nature involved in each response. In the current experiment, MCFA reduce viral replication by acting on MARC-145 cells, but not the virus, suggesting that there is an effect on PRRSV attachment or membrane fusion in the cytoplasm. Further studies are needed to understand the mechanism by which MCFA reduce PRRSV replication in MARC-145 cells.

In conclusion the results of this experiment confirm our previous results and demonstrate that MCFA combinations reduce viral replication in both PRRSV strains with MARC-145 cells. The mode of action of MCFA have been reported as an effect on bacterial membranes or viral envelopes. However, the results of this experiment do not demonstrate a direct effect on the PRRSV. Next steps would be to conduct additional research on the mode of action through which MCFA reduce viral replication of PRRSV and confirm these results in a PRRSV challenge with nursery pigs.

3.6 Literature Cited

Baltić, B., M. Starčević, J. Đorđević, B. Mrdović, and R. Marković. 2017. Importance of medium chain fatty acids in animal nutrition. IOP Conf. Ser. Earth Environ. Sci. 85:012048. doi:10.1088/1755-1315/85/1/012048. Available from: https://iopscience.iop.org/article/10.1088/1755-1315/85/1/012048

Briscoe, C. P., M. Tadayyon, J. L. Andrews, W. G. Benson, J. K. Chambers, M. M. Eilert, C. Ellis, N. A. Elshourbagy, A. S. Goetz, D. T. Minnick, P. R. Murdock, H. R. Sauls, U. Shabon, L. D. Spinage, J. C. Strum, P. G. Szekeres, K. B. Tan, J. M. Way, D. M. Ignar, S. Wilson, and A. I. Muir. 2003. The Orphan G Protein-coupled Receptor GPR40 Is Activated by Medium and Long Chain Fatty Acids. J. Biol. Chem. 278:11303–11311. doi:10.1074/jbc.M211495200. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925819324147

Calder, P. C. 2008. The relationship between the fatty acid composition of immune cells and their function. doi:10.1016/j.plefa.2008.09.016. Available from: www.elsevier.com/locate/plefa

De Baere, M. I., H. Van Gorp, P. L. Delputte, and H. J. Nauwynck. 2012. Interaction of the European genotype porcine reproductive and respiratory syndrome virus (PRRSV) with sialoadhesin (CD169/Siglec-1) inhibits alveolar macrophage phagocytosis. Vet. Res. 43:47. doi:10.1186/1297-9716-43-47. Available from: http://www.veterinaryresearch.org/content/43/1/47

Decuypere, J. A., and N. A. Dierick. 2003. The combined use of triacylglycerols containing medium-chain fatty acids and exogenous lipolytic enzymes as an alternative to in-feed antibiotics in piglets: concept, possibilities and limitations. An overview. Nutr. Res. Rev. 16:193–210. doi:10.1079/NRR200369. Available from: https://www.cambridge.org/core/product/identifier/S0954422403000143/type/journal_article

Delputte, P. L., W. Van Breedam, I. Delrue, C. Oetke, P. R. Crocker, and H. J. Nauwynck. 2007. Porcine Arterivirus Attachment to the Macrophage-Specific Receptor Sialoadhesin Is Dependent on the Sialic Acid-Binding Activity of the N-Terminal Immunoglobulin Domain of Sialoadhesin. J. Virol. 81:9546–9550. doi:10.1128/JVI.00569-07. Available from: https://jvi.asm.org/content/81/17/9546

Delputte, P. L., N. Vanderheijden, H. J. Nauwynck, and M. B. Pensaert. 2002. Involvement of the Matrix Protein in Attachment of Porcine Reproductive and Respiratory Syndrome Virus to a Heparinlike Receptor on Porcine Alveolar Macrophages. J. Virol. 76:4312–4320. doi:10.1128/JVI.76.9.4312-4320.2002. Available from: http://jvi.asm.org/

Desbois, A. P., and V. J. Smith. 2010. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl. Microbiol. Biotechnol. 85:1629–1642. doi:10.1007/s00253-009-2355-3. Available from: http://link.springer.com/10.1007/s00253-009-2355-3

Dichtelmüller, H., D. Rudnick, and M. Kloft. 2002. Inactivation of Lipid Enveloped Viruses by Octanoic Acid Treatment of Immunoglobulin Solution. Biologicals. 30:135–142. doi:10.1006/biol.2002.0332. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1045105602903325

Du, T., Y. Nan, S. Xiao, Q. Zhao, and E.-M. Zhou. 2017. Antiviral Strategies against PRRSV Infection. Trends Microbiol. 25:968–979. doi:10.1016/j.tim.2017.06.001. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0966842X17301348

Guo, C., Z. Zhu, Y. Guo, X. Wang, P. Yu, S. Xiao, Y. Chen, Y. Cao, and X. Liu. 2017. Heparanase Upregulation Contributes to Porcine Reproductive and Respiratory Syndrome Virus Release. J. K. Pfeiffer, editor. J. Virol. 91. doi:10.1128/JVI.00625-17. Available from: https://jvi.asm.org/lookup/doi/10.1128/JVI.00625-17

Haghikia, A., S. Jörg, A. Duscha, J. Berg, A. Manzel, A. Waschbisch, A. Hammer, D.-H. Lee, C. May, N. Wilck, A. Balogh, A. I. Ostermann, N. H. Schebb, D. A. Akkad, D. A. Grohme, M. Kleinewietfeld, S. Kempa, J. Thöne, S. Demir, D. N. Müller, R. Gold, and R. A. Linker. 2015. Dietary Fatty Acids Directly Impact Central Nervous System Autoimmunity via the Small Intestine. Immunity. 43:817–829. doi:10.1016/j.immuni.2015.09.007. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1074761315003921

Hanczakowska, E., M. Świątkiewicz, P. Hanczakowski, and A. Wróbel. 2010. Medium-chain fatty acids as feed supplements for weaned piglets. Med. Weter. 66:331–334.

Heaton, N. S., and G. Randall. 2011. Multifaceted roles for lipids in viral infection. Trends Microbiol. 19:368–375. doi:10.1016/j.tim.2011.03.007. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0966842X11000576

Hilmarsson, H., T. KRISTMUNDSDOTTIR, and H. THORMAR. 2005. Virucidal activities of medium- and long-chain fatty alcohols, fatty acids and monoglycerides against herpes simplex virus types 1 and 2: comparison at different pH levels. APMIS. 113:58–65. doi:10.1111/j.1600-0463.2005.apm1130109.x. Available from: http://doi.wiley.com/10.1111/j.1600-0463.2005.apm1130109.x

Holtkamp, D. J., J. B. Kliebenstein, E. J. Neumann, J. J. Zimmerman, H. F. Rotto, T. K. Yoder, C. Wang, P. E. Yeske, C. L. Mowrer, and C. A. Haley. 2013. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. Available from: http://www.aasv.org/shap.html.

Hsu, N.-Y., O. Ilnytska, G. Belov, M. Santiana, Y.-H. Chen, P. M. Takvorian, C. Pau, H. van der Schaar, N. Kaushik-Basu, T. Balla, C. E. Cameron, E. Ehrenfeld, F. J. M. van Kuppeveld, and N. Altan-Bonnet. 2010. Viral Reorganization of the Secretory Pathway Generates Distinct Organelles for RNA Replication. Cell. 141:799–811. doi:10.1016/j.cell.2010.03.050. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867410003697

Huang, L., Y.-P. Zhang, Y.-L. Yu, M.-X. Sun, C. Li, P.-Y. Chen, and X. Mao. 2011. Role of lipid rafts in porcine reproductive and respiratory syndrome virus infection in MARC-145 cells. doi:10.1016/j.bbrc.2011.09.109.

Jackman, J. A., A. Hakobyan, H. Zakaryan, and C. C. Elrod. 2020. Inhibition of African swine fever virus in liquid and feed by medium-chain fatty acids and glycerol monolaurate. J. Anim. Sci. Biotechnol. 11:114. doi:10.1186/s40104-020-00517-3. Available from: https://jasbsci.biomedcentral.com/articles/10.1186/s40104-020-00517-3

Jiang, Y., G. Li, L. Yu, L. Li, Y. Zhang, Y. Zhou, W. Tong, C. Liu, F. Gao, and G. Tong. 2020. Genetic Diversity of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) From 1996 to 2017 in China. Front. Microbiol. 11. doi:10.3389/fmicb.2020.00618. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2020.00618/full

Kabara, J. J., R. Vrable, and M. S. F. Lie Ken Jie. 1977. Antimicrobial lipids: Natural and synthetic fatty acids and monoglycerides. Lipids. 12:753–759. doi:10.1007/BF02570908. Available from: http://doi.wiley.com/10.1007/BF02570908

Kärber, G. 1931. Beitrag zur kollektiven Behandlung pharmakologischer. Beitrag zur Kollekt. Behandlung pharmakologischer Reihenversuche. 1–4.

Lee, C. 2015. Porcine epidemic diarrhea virus: An emerging and re-emerging epizootic swine virus. Virol. J. 12:193. doi:10.1186/s12985-015-0421-2. Available from: http://www.virologyj.com/content/12/1/193

Long, S., Y. Zhou, D. Bai, W. Hao, B. Zheng, S. Xiao, and L. Fang. 2019. Fatty Acids Regulate Porcine Reproductive and Respiratory Syndrome Virus Infection via the AMPK-ACC1 Signaling Pathway. doi:10.3390/v11121145. Available from: www.mdpi.com/journal/viruses

Lorizate, M., T. Sachsenheimer, B. Glass, A. Habermann, M. J. Gerl, H.-G. Kräusslich, and B. Brügger. 2013. Comparative lipidomics analysis of HIV-1 particles and their producer cell membrane in different cell lines. Cell. Microbiol. 15:292–304. doi:10.1111/cmi.12101. Available from: http://doi.wiley.com/10.1111/cmi.12101

Martínez-Vallespín, B., W. Vahjen, and J. Zentek. 2016. Effects of medium-chain fatty acids on the structure and immune response of IPEC-J2 cells. Cytotechnology. 68:1925–1936. doi:10.1007/s10616-016-0003-1. Available from: http://link.springer.com/10.1007/s10616-016-0003-1

Más, V., and J. A. Melero. 2013. Entry of enveloped viruses into host cells: Membrane fusion. Subcell. Biochem. 68:467–487. doi:10.1007/978-94-007-6552-8_16. Available from: https://pubmed.ncbi.nlm.nih.gov/23737062/

Mazzon, M., and J. Mercer. 2014. Lipid interactions during virus entry and infection. Cell. Microbiol. 16:1493–1502. doi:10.1111/cmi.12340. Available from: http://doi.wiley.com/10.1111/cmi.12340

Ono, A., and E. O. Freed. 2005. Role of Lipid Rafts in Virus Replication. In: p. 311–358. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0065352705640109

Schlievert, P. M., and M. L. Peterson. 2012. Glycerol Monolaurate Antibacterial Activity in Broth and Biofilm Cultures. G. F. Kaufmann, editor. PLoS One. 7:e40350. doi:10.1371/journal.pone.0040350. Available from: https://dx.plos.org/10.1371/journal.pone.0040350

Van Breedam, W., P. L. Delputte, H. Van Gorp, G. Misinzo, N. Vanderheijden, X. Duan, and H. J. Nauwynck. 2010. Porcine reproductive and respiratory syndrome virus entry into the porcine macrophage. J. Gen. Virol. 91:1659–1667. doi:10.1099/vir.0.020503-0. Available from: https://www.microbiologyresearch.org/content/journal/jgv/10.1099/vir.0.020503-0

Van Meer, G., D. R. Voelker, and G. W. Feigenson. 2008. Membrane lipids: where they are and how they behave. Nat. Rev. Mol. Cell Biol. 9:112–124. doi:10.1038/nrm2330. Available from: http://www.nature.com/articles/nrm2330

Wang, L. L., and E. A. Johnson. 1992. Inhibition of Listeria monocytogenes by fatty acids and monoglycerides. Appl. Environ. Microbiol. 58:624–629. doi:10.1128/AEM.58.2.624-629.1992. Available from: https://aem.asm.org/content/58/2/624

Ying, S., X. ShaoBo, W. Dang, L. Rui, L. Bin, C. HuanChun, and F. LiuRong. 2011. SCIENCE CHINA Cellular membrane cholesterol is required for porcine reproduc-tive and respiratory syndrome virus entry and release in MARC-145 cells. Sci China Life Sci. 54:1011–1018. doi:10.1007/s11427-011-4236-0. Available from: www.springer.com/scp

Yoon, B., J. Jackman, E. Valle-González, and N.-J. Cho. 2018. Antibacterial Free Fatty Acids and Monoglycerides: Biological Activities, Experimental Testing, and Therapeutic Applications. Int. J. Mol. Sci. 19:1114. doi:10.3390/ijms19041114. Available from: http://www.mdpi.com/1422-0067/19/4/1114

Zentek, J., S. Buchheit-Renko, F. Ferrara, W. Vahjen, A. G. Van Kessel, and R. Pieper. 2011. Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Anim. Health Res. Rev. 12:83–93. doi:10.1017/S1466252311000089.

Zhang, M. S., A. Sandouk, and J. C. D. Houtman. 2016. Glycerol Monolaurate (GML) inhibits human T cell signaling and function by disrupting lipid dynamics. Sci. Rep. 6:30225. doi:10.1038/srep30225. Available from: http://www.nature.com/articles/srep30225

Zhang, M. S., P. M. Tran, A. J. Wolff, M. M. Tremblay, M. G. Fosdick, and J. C. D. Houtman. 2018. Glycerol monolaurate induces filopodia formation by disrupting the association between LAT and SLP-76 microclusters. Sci. Signal. 11:eaam9095. doi:10.1126/scisignal.aam9095. Available from: https://stke.sciencemag.org/lookup/doi/10.1126/scisignal.aam9095

Figure 3.1. Incubation of MARC-145 cells with C8:C10 prior to and after inoculation with Type II North American (P129) PRRSV resulted in 2.2, 3.3, 4.1, and 4.0 log reductions ($P < 0.05$) in PRRSV replication at 200, 300, 400, and 500 µg/mL concentrations respectively as indicated by Log_{10} $TCID_{50}$/mL. [abc] Letters indicate differences ($P < 0.05$).
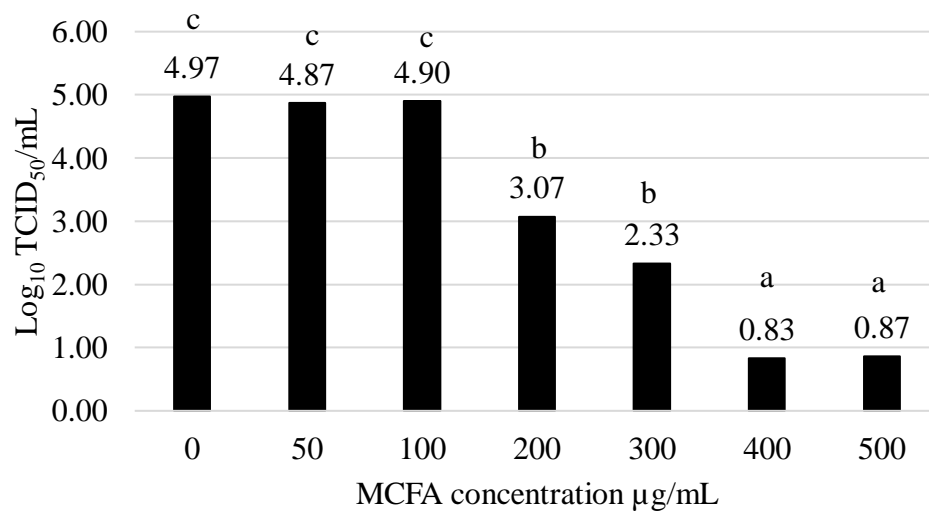
Figure 3.2. Incubation of MARC-145 cells with C8:10 prior to and after inoculation with Type I European (Lelystad) PRRSV resulted in 1.9, 2.6, 4.1, and 4.1 log reductions ($P < 0.05$) in PRRSV replication at 200, 300, 400, and 500 µg/mL concentrations respectively as indicated by Log_{10} $TCID_{50}$/mL. [abc] Letters indicate differences ($P < 0.05$).
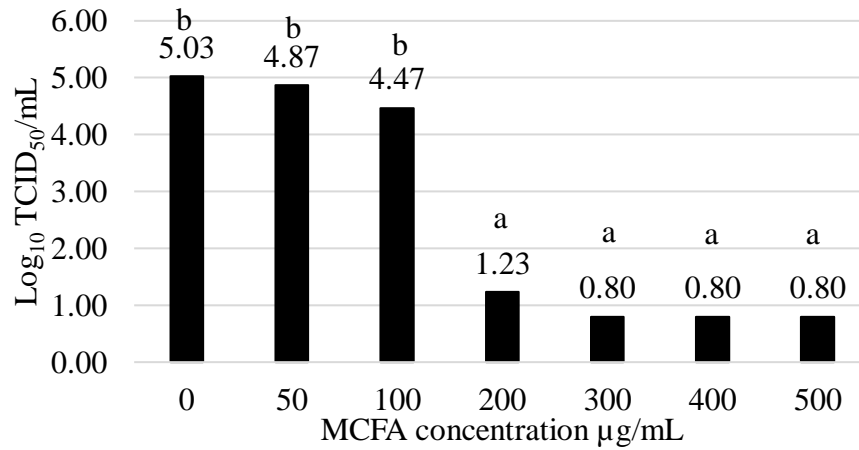
Figure 3.3 Incubation of MARC-145 cells with C8:12 prior to and after inoculation with Type II North American (P129) PRRSV resulted in 3.8 log reduction ($P < 0.05$) in PRRSV replication at the 200 µg/mL concentration and 4.2 log reduction ($P < 0.05$)in PRRSV replication at 300, 400, and 500 µg/mL concentrations as indicated by Log_{10} $TCID_{50}$/mL.

[ab] Letter indicate differences ($P < 0.05$)
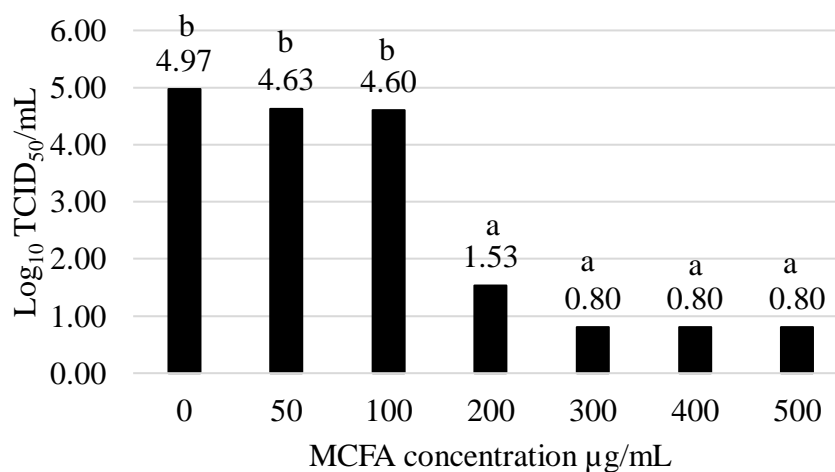
Figure 3.4 Incubation of MARC-145 cells with C8:12 prior to and after inoculation with Type I European (Lelystad) PRRSV resulted in 3.4 log reduction ($P < 0.05$) in PRRSV replication at the 200 µg/mL concentration and 4.2 log reduction ($P < 0.05$) in PRRSV replication at 300, 400, and 500 µg/mL concentrations as indicated by $\log_{10}$ (TCID$_{50}$)/mL. [ab] Letters indicate differences ($P < 0.05$).
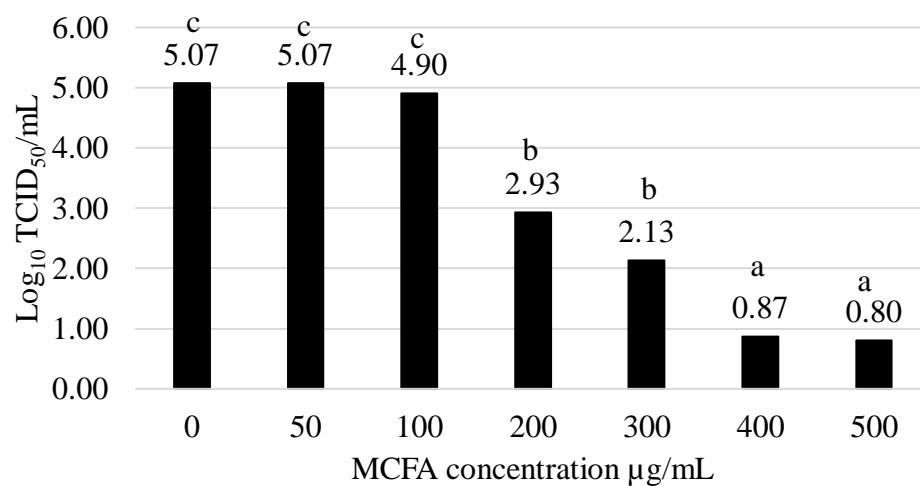
Figure 3.5 Incubation of MARC-145 cells with C10:12 prior to and after inoculation with Type II North American (P129) PRRSV resulted in 2.1, 2.9, 4.2, and 4.3 log reductions ($P < 0.05$) in PRRSV replication at 200, 300, 400, and 500 µg/mL concentrations as indicated by Log_{10} $TCID_{50}$/mL. [abc] Letters indicate differences ($P < 0.05$).
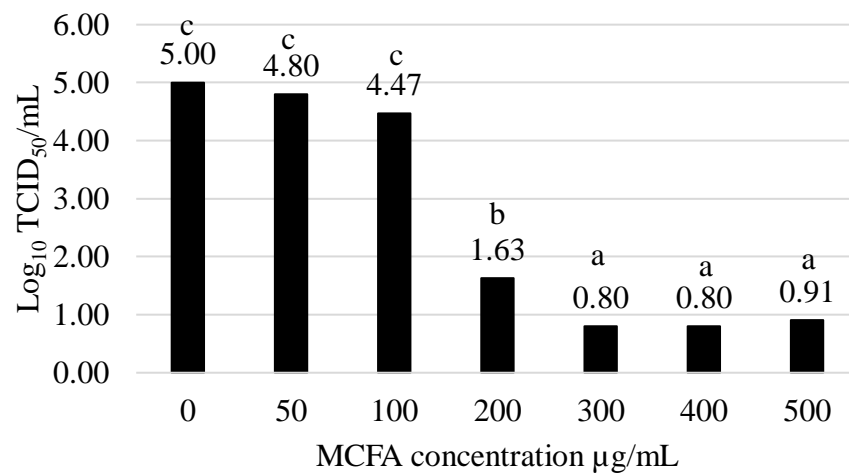
Figure 3.6 Incubation of MARC-145 cells with C10:12 prior to and after inoculation with Type I European (Lelystad) PRRSV resulted in 3.37, 4.2, 4.2, and 4.1 log reductions ($P < 0.05$) in PRRSV replication at the 200, 300, 400, and 500 µg/mL concentrations respectively as indicated by $\log_{10}$ ($TCID_{50}$)/mL. [abc] Letters indicate differences ($P < 0.05$).
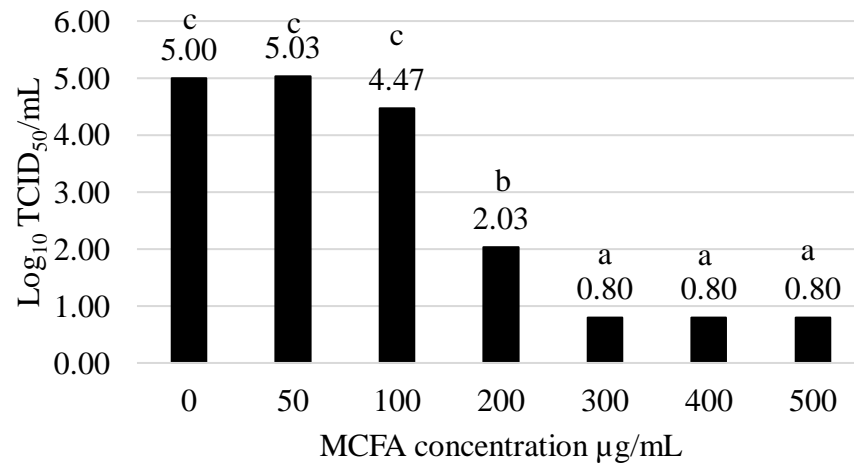
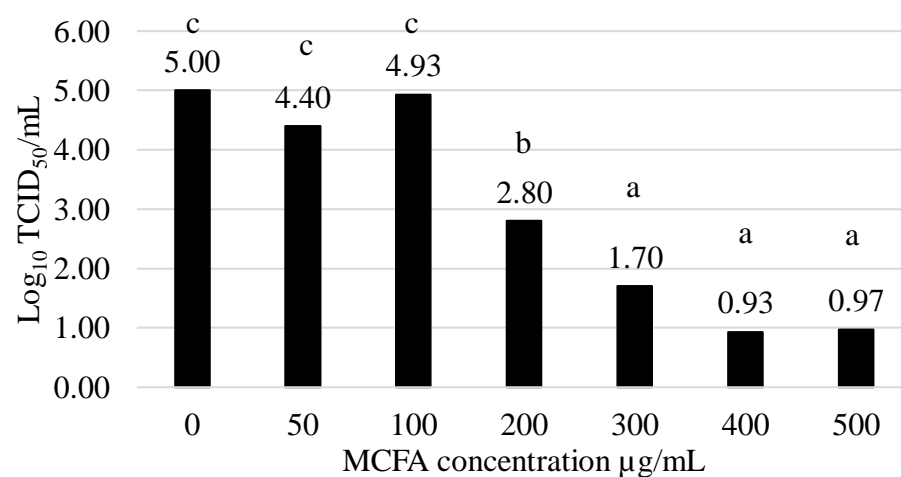Figure 3.7 Incubation of MARC-145 cells with C8:C10:C12 prior to and after inoculation with Type II North American (P129) PRRSV resulted in 3.0 log reduction ($P < 0.01$) in PRRSV replication at 200 µg/mL concentration and 4.2 log reduction ($P < 0.01$) in PRRSV replication at 300, 400, and 500 µg/mL concentrations as indicated by Log_{10} $TCID_{50}$/mL [abc] Letters indicate differences ($P < 0.01$).
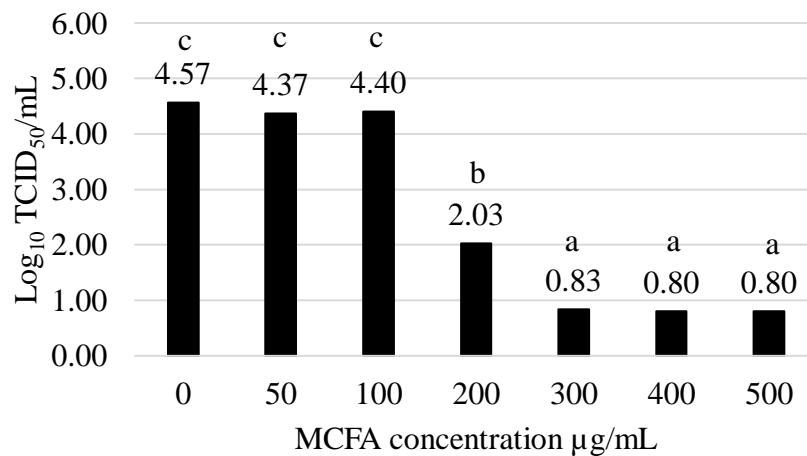
Figure 3.8 Incubation of MARC-145 cells with C8:C10:C12 prior to and after inoculation with Type I European (Lelystad) PRRSV resulted in 2.5 and 3.7 log reductions ($P < 0.01$) in PRRSV replication at 200 and 300 µg/mL concentrations respectively. The 400 and 500 µg/mL concentrations resulted in 3.8 log reduction ($P < 0.01$) in PRRSV replication as indicated by Log_{10} $TCID_{50}$/mL. [abc] Letters indicate differences ($P < 0.01$).
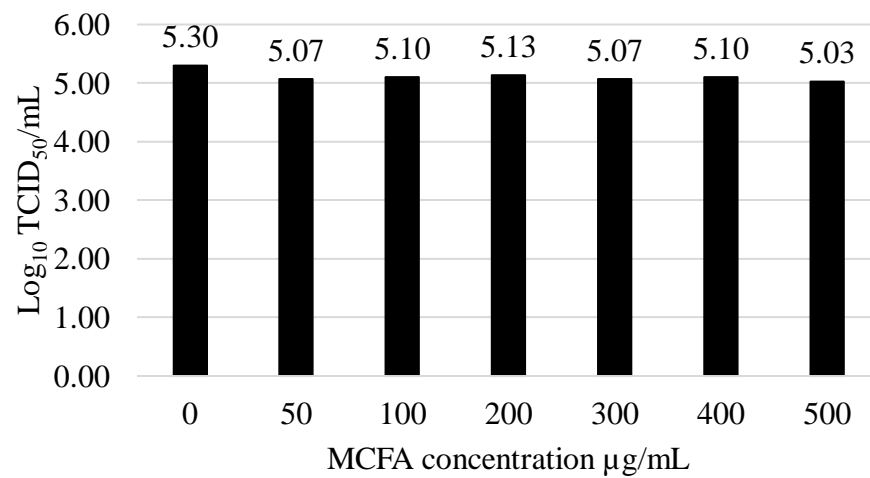
Figure 3.9 Treatment of Type II North American (P129) PRRSV with C8:C10:C12 prior to inoculation of MARC-145 cells with no MCFA resulted in no reduction ($P > 0.05$) in PRRSV replication as indicated by Log_{10} $TCID_{50}$/mL.
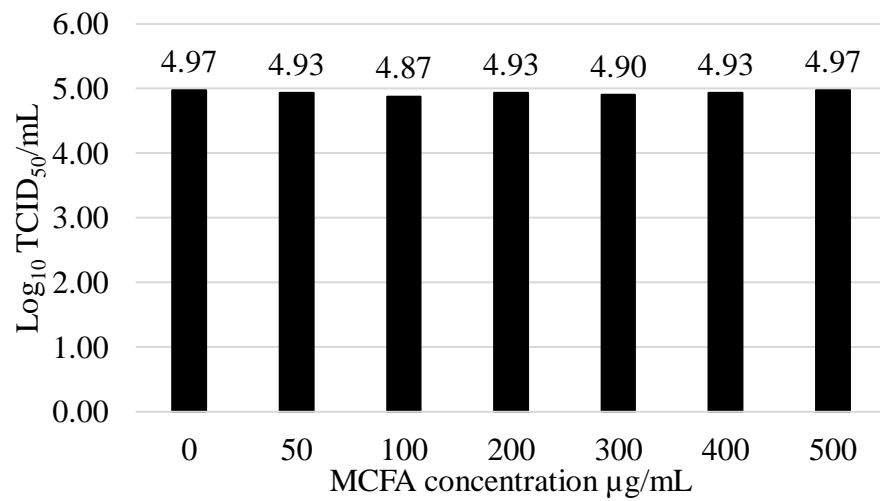
Figure 3.10. Treatment of Type II North American (P129) PRRSV with C8:C10:C12 prior to inoculation of MARC-145 cells with no MCFA resulted in no reduction ($P > 0.05$) in PRRSV replication as indicated by Log_{10} $TCID_{50}$/mL.
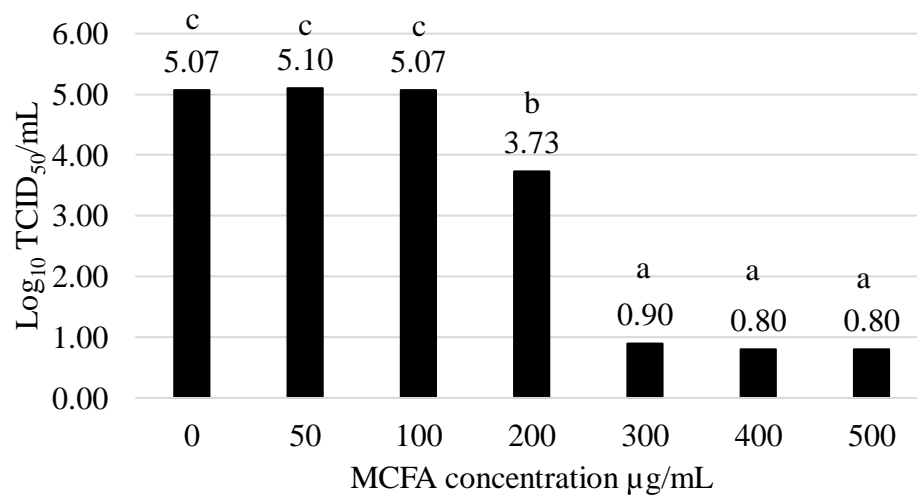
Figure 3.11. MARC-145 incubated with C8:C10:C12 prior to and after inoculation with Type II North American (P129) PRRSV resulted in 1.3 and 4.2 log reductions ($P < 0.01$) in PRRSV replication at 200 and 300 µg/mL concentrations respectively. The 400 and 500 µg/mL concentrations resulted in 4.3 log reduction ($P < 0.01$) in PRRSV replication as indicated by Log_{10} $TCID_{50}$/ml [abc] Letters indicate differences ($P < 0.01$).
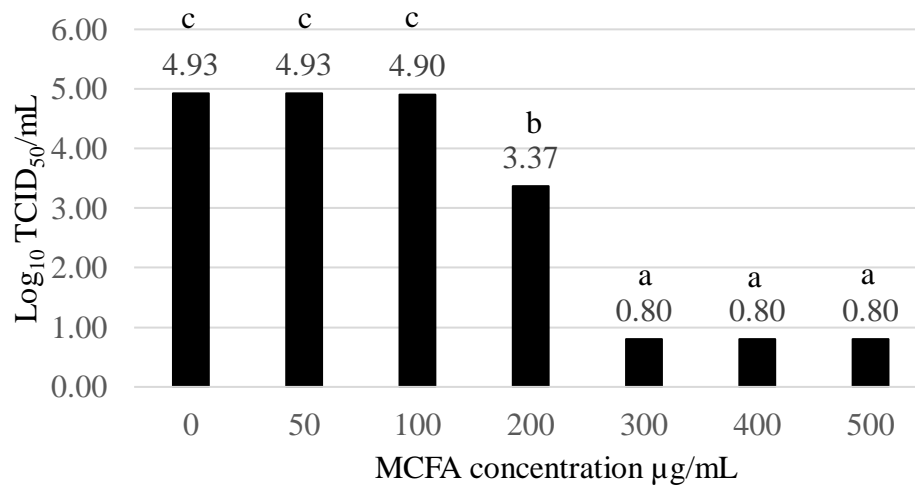
Figure 3.12. MARC-145 incubated with C8:C10:C12 prior to and after inoculation with Type I European (Lelystad) PRRSV resulted in a 1.6 log reduction ($P < 0.01$) in PRRSV replication at 200 µg/mL concentration and a 4.0 log reduction ($P < 0.01$) in PRRSV replication at 300, 400 and 500 µg/ml concentrations as indicated by Log_{10} $TCID_{50}$/mL. [abc] Letters indicate differences ($P < 0.01$).

CHAPTER 4.   THE EFFECT OF MCFA FED TO PIGS ON PRRSV INFECTION

4.1   Abstract

One hundred twelve mixed sex pigs (PIC 1050 females x PIC 359 sire) weaned at 21 d of age (average weight 7.5 ±0.68kg) were used in a 33 d PRRSV challenge experiment. Pigs were acclimated to the facility and diets for 5 d before inoculation with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). Pigs were inoculated with either a placebo (sterile PBS) or Type II North American (P129) PRRSV (1 x 10^5, $TCID_{50}$/mL) given in 1 mL each intranasal and IM injection. Pigs were blocked by body weight and sex and randomly allotted to one of four treatment rooms in a 2x2 factorial design with pigs receiving 0 or 0.30% MCFA in the diet and receiving placebo or PRRSV inoculation. Each room contained 4 treatment pens with 7 pigs per pen with an equal ratio of barrows to gilts within treatment. Diets were formulated to meet or exceed all nutritional requirements (NRC, 2012) and were fed in 4 nursery phases. Feed budgets by phase were 1.13 kg/pig in phase 1, 2.72 kg/pig in phase 2, 6.35 kg/pig in phase 3, and phase 4 was fed until the end of the experiment. MCFA (C8:C12) was mixed in a 1:1 ratio (wt:wt) with finely ground corn to prepare a premix that was added to the diet at 0.60% to provide 0.30% total MCFA. Control diets used soybean oil mixed with finely ground corn at the same 0.60% inclusion to keep metabolizable energy levels constant across treatments. Body weights, feed intake, blood samples, and temperatures were determined on d 0, 3, 7, 10, 14, 21, and 28 post-inoculation. Sections of tonsil, lung, and intestines were collected at d 10 post-inoculation from 1 pig per pen and at d 28 from all remaining pigs. Data were analyzed as a 2 x 2 factorial (PRRSV infection x MCFA) using the PROC Mixed procedure of SAS with pen as the experimental unit for growth performance measurements and pig as the experimental unit for viral load analysis. Serum viral

load confirmed PRRSV was only detectable in challenged pigs. Body weights were not different ($P > 0.05$) between treatments prior to d 14 post-inoculation. Body weights from d 14 to 28 were reduced ($P < 0.05$) in PRRSV infected pigs when compared to non-infected pigs. Overall ADG and ADFI was reduced ($P < 0.05$) for PRRSV infected pigs compared to non-infected pigs by an average of 18 and 28%, respectively. Body temperatures were not different between treatments. Viral load measured in the lung was not different ($P > 0.05$) between PRRSV infected treatments. Tonsil viral load was not different ($P > 0.10$) between PRRSV treatments. However, there was a trend ($P \leq 0.10$) for an effect of day post inoculation with control fed PRRSV infected pigs having higher viral loads at d 10 post-inoculation compared to d 28 post-inoculation. Overall, PRRSV infection reduced performance in this experiment when compared to non-infected pigs and there was no effect of diet on PRRSV viral load or performance in this experiment.

4.2 Introduction

The swine industry has been impacted by Porcine Reproductive Respiratory Syndrome Virus (PRRSV) for over 30 years (Brar et al., 2014). First appearance of the virus occurred in the late 1980s in the US and Europe as a mystery swine disease. Virus isolation in 1991 in Europe and 1992 in the US lead to the identification of 2 PRRSV genotypes: the type I European and type II North American. PRRSV is an enveloped, positive single strand RNA virus in the Arteriviridae family of viruses in the order of Nidovirales. Transmission of the virus is both vertical (sow to piglet) and horizontal (pig to pig) by nasopharyngeal and by aerosol routes. PRRSV infection results in reproductive failure in sows and gilts as well as respiratory illness in all phases of pig production. Reproductive failure in sows can range from decreased farrowing rates to increased abortions and low viability in liveborn pigs. Respiratory disease severity increases with the presence of secondary infections leading to high mortality rates in pre- and post-weaned pigs. It

has been estimated that PRRSV infection annually costs the US Pork Industry $664 million in production losses making it the most economically significant disease facing today's pork producers (Holtkamp et al., 2013). Approximately 55% of the economic losses are due to high mortality, decreased growth rates, and respiratory distress in growing pigs

Over 30 years of research on the pathogenesis of PRRSV and mechanisms to long-term immunity and cross protection are still unclear. Challenges to PRRSV research and subsequent control and prevention includes the fast mutation rate, viral modulation of the immune response, the persistence of infection after known symptoms are gone, and rapid transmission or spread to other animals and locations. Genetic diversity between PRRSV strains has led to inadequate vaccine efficacy at generating long term immunity and cross protection. The delay in neutralizing antibodies to the PRRSV has contributed to evasion of the host immune response and persistence in a herd. Decoy epitopes on the PRRSV are contributing factors to the delayed presence of neutralizing antibodies. PRRSV suppresses Type I Interferon (INFα) in evading the host immune response. Research has reported that INFα has inhibited PRRSV infection (Albina et al., 1998). Even with evasion of the immune response, the pig will produce neutralizing antibodies 4-8 weeks post-infection, which indicates that the mechanism of suppression is not long lasting. PRRSV management and prevention programs have focused on biosecurity and limiting animal movement with vaccination strategies. However, even with these management steps the lack of cross protection and persistence of infection increase economic losses for producers with each recurring break of PRRSV in a herd.

This has brought about increased interest in research focused on nutritional implications or strategies to improve animal health. Nutritional impacts on health require intercommunication between gut tissue, the central nervous system, and the immune system (Rehfeld, 2004; Janssen &

Depoortere, 2013). Communication between the gut and other organ immune functions such as Gut-Lung, Gut-Brain, Gut-Liver provide evidence to support the influence of nutrition on immune responses (Xiao et al., 2011; Furness et al., 2013; Budden et al., 2017; Ge et al., 2021).

MCFA have emerged as a target feed additive to improve animal health and performance (Cochrane et al., 2016; Gebhardt et al., 2017). MCFA can shift microbial populations by inhibiting pathogenic bacteria and thereby allowing an increased growth of beneficial bacteria (Yoon et al., 2018). This knowledge provides the foundation that nutrition can impact animal health and performance. Research on intestinal morphology and gut microbiota have reported an increase in intestinal villi height and crypt depth as well as modified microbiota with the use of MCFA in pig diets (Dierick et al., 2003; Calder, 2008; Zentek et al., 2012). Understanding the vast interaction of the gut with other regulatory systems is still not complete but does provide new targets for impacting health and performance in the animal as well as new targets for therapeutic products to prevent infection and disease spread.

Viral transmission through feed has been of increased interest following the emergence of Porcine Epidemic Diarrhea Virus (PEDV) in 2013. Research on feed mitigation has reported that MCFA are effective at reducing viral particles and infectivity of feed and feed ingredients (Lerner et al., 2020). These findings suggest that it is possible to influence animal health and the immune response through nutrition. These finding coupled with our prior in vitro results have led to the hypothesis that MCFA fed to pigs can inhibit or reduce viral infection, thereby decreasing disease duration and/or persistence. Therefore, the objective of this experiment was to feed MCFA to pigs and then challenge with PRRSV to determine the antiviral activity of MCFA in vivo.

4.3 Materials and Methods

4.3.1 General

This was the third experiment in a series to test our overall hypothesis that providing MCFA to pigs through feed would inhibit or reduce PRRSV infection. This experiment was designed to test the combination of MCFA C8:C12 (1:1; wt:wt) on the effectiveness to reduce PRRSV infection in pigs.

Data were analyzed as a 2x2 factorial using the PROC Mixed procedure of SAS (SAS Institute INC., Cary, NC) with pen as the experimental unit for growth performance measurements and pig as the experimental unit for viral load analysis. Data are presented as LS means. Statistical significance was defined as $P \leq 0.05$ and a tendency was defined as $0.05 < P \leq 0.10$.

4.3.2 Animals

One hundred twelve mixed sex pigs (PIC 1050 females x PIC 359 sire), weaned at 21 d of age weighing 7.51 ±0.68 kg, were used in a 33 d PRRSV challenge experiment. Pigs were blocked by body weight and sex and randomly assigned to one of four treatment rooms in a 2x2 factorial design with pigs receiving 0 or 0.30% MCFA in the diet and receiving placebo (sterile PBS) or PRRSV inoculation. Each room contained 4 treatment pens with 7 pigs per pen with an equal ratio of barrows to gilts within treatment. Pigs were acclimated to the facility and diets for 5 d before PRRSV inoculation. All procedures were approved by the Carthage Innovative Swine Solutions (CISS) research animal care and use committee. The experiment was conducted at the CISS, LLC BSL-2 facility, Champaign IL. Daily health observations were taken, and no pigs were treated or removed due to health abnormalities during the experiment.

4.3.3 Diets

Each room was designated to one of four treatments: 1) control-uninfected, 2) control PRRSV-infected, 3) MCFA-uninfected, and 4) MCFA-PRRSV infected. Diets were formulated to meet or exceed all nutritional requirements (NRC, 2012) and were fed in 4 nursery phases (Table 4.1 and Table 4.2). Feed budgets were 1.13 kg/pig in phase 1, 2.72 kg/pig in phase 2, 6.35 kg/pig in phase 3, and phase 4 was fed until the end of the experiment. MCFA (C8:C12) were mixed in a 1:1 ratio (wt:wt) then added to a premix with finely ground corn to be mixed into diets at 0.60% to provide 0.30% total MCFA. Control diets used soybean oil mixed with finely ground corn at the same 0.60% inclusion to keep ME concentrations constant across treatments. Pigs had ad libitum access to feed and water throughout the experiment.

4.3.4 Measurements and Sample Collection

Individual body weights, blood samples, rectal temperatures, and pen feed intakes were determined at d 0, 3, 7, 10, 14, 21, and 28 post-inoculation. Blood samples were collected for analysis of serum viral load in a serum separator vacutainer (BD Vacutainer #367985). Serum was separated and stored in 1.5 mL microcentrifuge tubes and stored at -80ºC until RNA extraction.

One pig from each pen was euthanized for tissue collection at d 10 post-inoculation and remaining pigs were euthanized d 28 post-inoculation. Euthanasia was by captive bolt, followed by exsanguination. Viscera were then removed from the body cavity. Sections of tonsil and lung were collected and placed in 2 mL cryo-vials, flash frozen in liquid nitrogen, and stored at -80ºC for subsequent RNA extraction. The intestinal tract was separated to dissect 15 cm segments from the duodenum, jejunum, and ileum. Intestinal tissue was rinsed with phosphate buffered saline (PBS) to remove digesta. Intestinal tissues were cut longitudinally along the mesenteric boarder to expose the inner mucosa. Mucosa was scrapped using the edge of a glass microscope slide (24.5

mm x 76.2 mm), and scrapings were deposited into 2 mL cryo-vials, flash frozen in liquid nitrogen, and stored at -80ºC for subsequent RNA extraction. Intestinal tissue sub-sections were placed in histology cassettes (Tissue-Tek Uni-cassettes; Sakura Finetek USA, Inc., Torrance CA) and stored in 10% neutral buffered formalin solution for subsequent histological analysis.

4.3.5 Sample analysis

Serum RNA was extracted using the Purelink Kit (Invitrogen #12280050 ThermoFisher Scientific, Life Technologies Corporation, Carlsbad, CA 92008). Briefly, 200 µL of serum was added to a 1.5 mL microcentrifuge tube with 25 µL of proteinase K and 200 µL of lysis buffer containing 5.6 µL of carrier RNA. Samples were incubated at 56 ºC for 15 min and then 250 µL of 100% ethanol was added, followed by a 5 min incubation at room temperature. The sample was then transferred to a viral spin column with collection tube and centrifuged at 7,000 x g for 1 min. Following centrifugation, the sample was washed twice using a wash buffer and re-centrifuged at 7,000 x g for 1 min. The sample was then transferred to a dry spin column and centrifuged at 18,000 x g for 2 min to dry. Following centrifugation, the spin column was placed in a 1.5 mL microcentrifuge recovery tube and 20 µL of nuclease free water was added directly to the membrane followed by a 2 min incubation at room temperature. The sample was then centrifuged at 16,000 x g for 2 min to elute RNA. RNA was stored at -80°C until subsequent qPCR analysis.

Tonsil and lung tissue were ground with a mortar and pestle using liquid nitrogen and 100 g of ground tissue was added to a 1.5 mL microcentrifuge tube and stored at -80ºC for subsequent RNA extraction. Tissue RNA was extracted using a column free extraction method (RNeasy extraction Qiagen Inc.). Briefly, ground tissue samples were removed from the -80°C freezer and placed on dry ice, 1 mL of Trizol was added, and then shaken vigorously by hand for 5 min. Immediately following, 200 µL of chloroform was added and then shaken vigorously by hand for

2 min. Samples were then centrifuged at 14,000 x g for 15 min and maintained at 4 °C. Carefully, so not to disturb phase separation, samples were removed from the centrifuge and placed on ice. The top clear aqueous layer was then removed and placed in a clean 1.5 mL microcentrifuge tube. An equal amount (~600 µL) of ice-cold isopropanol was added and mixed by inversion followed by a 45 min incubation on ice. Following incubation, samples were centrifuged at 14,000 x g for 15 min and maintained at 4 °C. The supernatant was removed and discarded, being careful not to disturb the pellet at the bottom of the tube. The pellet was then washed twice with 1 mL of ice cold 70% ethanol and centrifuged at 4 ℃ at 14,000 x g for 15 min. The supernatant was discarded after each wash. Following the second wash, the pellet was re-suspended in 200 µL of nuclease free water, 10 µL of 3M sodium acetate, and 200 µL of ice-cold isopropanol mixed by inversion and incubated overnight at -20 ℃. Samples were then removed from -20 °C storage and centrifuged at 4 ℃ at 14,000 x g for 15 min. The supernatant was carefully removed to not disturb the pellet at the bottom of the tube, and the pellet was washed by adding 1 ml of ice cold 70% ethanol and centrifuge at 4 ℃ at 14,000 x g for 15 min and supernatant was removed. The pellet was re-suspended in 30-50 µL of nuclease free water dependent on the size of the pellet. Samples were then quantified on a Nanodrop ND-1000 18 Spectrophotometer (Nanodrop Technologies, USA) at 260 nm wavelength and diluted with nuclease free water until concentration was under 2000 ng/µL. Sample 260:280 ratios were used as quality check to denote level of contamination. RNA samples were stored at -80℃ until DNase treatment.

DNase was used to remove any DNA from the sample and was completed using the reagents from the Turbo DNase Kit. RNA samples were removed from -80°C, placed on ice, and 20 µL of each RNA sample was transfered to a 0.5 mL microcentrifuge tube. Then 5 µL of mastermix (containing 2.5 µL 10X TurboDNase buffer, 1.5 µl nuclease free water, 0.5 µL Turbo

DNase, and 0.5 µL RNase OUT) was added, followed by a 20 min incubation at 37ºC. Immediately following incubation, 2.5 µL of DNase Inactivation Reagent was added and mixed by pipetting, followed by a 5 min incubation at room temperature with constant mixing. Following incubation, samples were centrifuged at 10,000 x g for 2 min, and then 20 µL of supernatant were transferred to a new 0.5 mL microcentrifuge tube careful not disturb the white pellet. RNA was quantified on a Nanodrop ND-1000 18 Spectrophotometer at 260 nm wavelength (Nanodrop Technologies, USA) and diluted with nuclease free water until concentration was under 1000 ng/µL. Sample 260:280 ratios were used as quality check to denote level of contamination. RNA samples post-DNase treatment were stored at -80ºC until reverse transcriptase and qPCR analysis. RNA integrity analysis was validated with 4 µg of each RNA sample using electrophoresis on 1% agarose gel with SyBr Safe, and the presences of two distinct bands, 18S and 28S, indicated that RNA extraction was successful.

Reverse transcription was accomplished using reagent from the High Capacity cDNA Reverse Transcriptase Kit. Post-DNase RNA samples were removed from -80°C storage and placed on ice. Specific volumes of each sample to provide 2000 ng of total RNA were calculated from the Nanodrop data. Mastermix was prepared with 3.7 µL of nuclease free water, 2.0 µL of 10X RT buffer, 2.0 µL of random primers, 0.8 µL of 25X dNTP [100mM], 1.0 µL of Multi Scribe Reverse Transcriptase, and 0.5 µL RNase OUT. Nuclease free water was added into RNase free strip tubes with each calculated RNA sample volume equal to 10µL total volume. 10 µL of mastermix was added to each sample tube and then tubes were capped. Tubes were quick spun to collect all liquid in the bottom, mixed by flicking, and quick spun again. Tubes were transferred to a thermocycler and run at 25 ºC for 10 min, 37 ºC for 120 min, 85 ºC for 5 min, and held at 4 ºC. Tubes were quick spun again to collect cDNA at the bottom of the tubes and cDNA was diluted

to 10 ng/µL equivalent RNA input with nuclease free water. Samples of cDNA were stored in at -20ºC until qPCR analysis.

A one-step qPCR using a PRRSV-2 specific primer-probe pair was conducted with quantification relative to a standard curve comprised of linearized topo plasmid 120 containing the targeted region of ORF5. One step qPCR conducted with 7.5 µL of SsoAdvanced Universal Probes Supermix (BioRad laboratories, #1725284), 0.50 µL of each forward and reverse primer (Table 4.3; manufactured by Integrated DNA Technologies, Inc., Coralville, IA), 4.5 µL of nuclease free water, and 2 µL of cDNA template. Ribosomal protein L 19 (RPL19) was utilized as the housekeeping gene. The PRRSV copy number values were normalized, for each sample per 100 copies RPL19.

Cytokine analysis was conducted using a sandwich ELISA assay. Flat bottom high binding ELISA plates (Greiner Bio) were coated overnight at 4°C with target specific antibodies using 100 µL of a 1:1000 dilution of the primary antibody (INFα #PB0157S and INFγ #KP1122S) in carbonate/bicarbonate coating buffer. All washing steps were carried out with 200 µL Tris buffered saline (TBS) + 0.1% Tween 20 (TBST) and plates were blocked with 0.1% BSA in wash buffer for 45 min at room temperature. Target capture from 100 µL serum samples, run in duplicate, were carried out at room temperature for 2 h. Target specific biotinylated detection antibodies were added at 100 µL of a 1:2000 dilution of secondary antibody (INFα #PBB0157S and IFNγ #KPB112S) and incubated for 2 h at room temperature. Colorimetric detection was carried out using 100 µL of streptavidin-HRP (R&D Systems #DY998) at 1:200 dilution and incubated for 45 min at room temperature, and then 100 µL of TMB (3,3'5,5'-tetramethylbenzidine) substrate was added followed by a 30 min incubation at room temperature. The enzymatic activity was stopped with 2M H_2SO_4 and absorption was measured at 450 nm using a microplate reader (Synergy LX,

multi-mode reader, BioTek) with quantity determined from the blank corrected OD using a 6-point standard curve. Intra-assay variation was determined using an inter-plate control.

Histology samples were processed by the Purdue Histology Research Laboratory at the Purdue University Veterinary School. The 10% neutral buffered formalin fixed tissue was embedded in paraffin, and a 5 mm thick section was sliced, and stained for hematoxylin and eosin (H&E). Slide images were obtained using an AmScope Microscope Digital Camera (MU1000, AmScope, Irvine, CA) at 40X magnification. Image J software (NIH, Nethesda, MD) was used to measure villi height, mid width, base width, and crypt depth on a minimum of 6 villi/crypt per slide.

4.4 Results

Body weights did not differ between treatments prior to d 14 post-inoculation (Table 4.4). PRRSV infection resulted in a 7% reduction in body weight on d 14 post-inoculation ($P \leq 0.05$). Body weights on d 21 post-inoculation were reduced ($P <0.05$) by PRRSV infection by 2.2 and 2.0 kg in control- and MCFA- fed pigs, respectively. PRRSV infection continued to reduce body weights on d 28 post-inoculation with PRRSV infected pigs weighing 2.6 kg less than uninfected pigs ($P < 0.05$). There was no effect of MCFA or MCFA*PRRSV on body weights in this experiment ($P > 0.05$).

Growth performance was not affected until after d 3 post-inoculation (Table 4.5). Overall, growth data is sporadic in the early stages of the experiment as pigs were weighed every 3-4 d. ADG from d 3-7 post inoculation was not different between treatments. PRRSV infection resulted in a 0.24 and 0.14 kg reduction ($P < 0.05$) in d 3-7 ADFI for control- and MCFA-fed pigs compared to their respective uninfected pigs. There was also a 0.16 kg reduction ($P < 0.05$) in ADFI for

MCFA-fed uninfected pigs compared to control-fed uninfected pigs from d 3-7. With these differences, G:F was greater for PRRSV infected pigs compared to uninfected pigs ($P < 0.05$).

ADG tended to be decreased ($P < 0.05$) with PRRSV infection from d 7-10 post-inoculation. There was an interaction of PRRSV and MCFA on ADFI from d 7-10 post-inoculation with PRRSV infection reducing ($P < 0.05$) ADFI in MCFA-fed pigs but not in control-fed pigs. There was an interaction ($P < 0.05$) of PRRSV and MCFA on G:F with MCFA-fed PRRSV infected pigs having the highest G:F and PRRSV infected control-fed pigs having the lowest G:F. ADG from d 10-14 post-inoculation was reduced ($P < 0.05$) with PRRSV infection and MCFA-fed pigs tended ($P < 0.10$) to have reduced ADG. There was an interaction ($P < 0.05$) of PRRSV infection and MCFA on ADFI from d 10-14 post-inoculation as result of lower intake in MCFA-fed pigs coupled with a greater reduction in ADFI observed in control-fed PRRSV infected pigs compared to MCFA-fed PRRSV infected pigs. There was a tendency ($P < 0.06$) for an interaction of PRRS infection and MCFA on G:F from d 10-14 post-inoculation. Control-fed uninfected pigs had the poorest G:F with control-fed PRRSV infected pigs having the highest G:F, and MCFA-fed pigs being intermediate.

PRRSV infection from d 14-21 resulted in a 26 and 30% decrease ($P < 0.01$) in ADG and ADFI, respectively, for control- and MCFA-fed pigs. There was no effect of PRRSV or MCFA on G:F during this time period. On d 21-28 PRRSV infected pigs tended ($P<0.10$) to have reduced ADG. ADFI was reduced ($P < 0.05$) by PRRSV infection and MCFA supplementation from d 21-28. There was an interaction of PRRSV and MCFA on G:F during d 21-28 post-inoculation with MCFA-fed PRRSV infected pigs having the greatest G:F ($P < 0.05$).

Overall ADG from d 0-28 post inoculation was 18.5% lower for PRRSV infected pigs in both control- and MCFA-fed pigs. PRRSV infection resulted in reduced ($P < 0.01$) ADFI of 25

and 35% in control- and MCFA-fed pigs, respectively. MCFA supplementation reduced ADFI during from d 0-28. Overall, there was a tendency for an interaction of PRRSV and MCFA on G:F. MCFA supplementation improved ($P < 0.05$) G:F in both uninfected and infected pigs but had a greater response in PRRSV infected pigs.

Rectal temperatures were not affected by PRRSV infection from d 0-10 post inoculation in this experiment (Figure 4.1). There was an effect of MCFA ($P < 0.05$) on d 14 post inoculation with MCFA-fed pigs having lower rectal temperatures than control-fed pigs. There was an interaction ($P < 0.05$) at d 21 post-inoculation for PRRSV and MCFA with the MCFA-fed PRRSV infected pigs having the lowest rectal temperature and MCFA-fed uninfected pigs having the highest temperature. On d 28 post-inoculation MCFA-fed pigs had higher ($P < 0.05$) rectal temperatures than control-fed pigs.

Serum viral concentrations (Figure 4.2) are reported as $\log_{10}$ of Type II North American (P129) PRRSV copies. All pigs were negative at d 0 of the experiment and uninfected pigs remained negative throughout the experiment. PRRSV infection increased serum viral concentrations in PRRSV inoculated pigs indicating successful inoculation with PRRSV. Serum viral concentrations were detected in PRRSV inoculated pigs at d 3 post-inoculation, and peaked between d 10 to 14 post-inoculation for both control- and MCFA-fed PRRSV infected pigs. Control- and MCFA-fed PRRSV infected pigs' serum viral concentrations were reduced after d 14 post-inoculation and continued to decrease to d 28 post-inoculation. There was no effect of MCFA on PRRSV serum concentrations. Area under the curve analysis detected that viral concentrations peaked at d 10 post inoculation in MCFA-fed PRRSV infected pigs and on d 14 post inoculation for control-fed PRRSV infected pigs (Figure 4.3). There was no significant

difference detected in area under the curve analysis for serum viral load between PRRSV infected pigs.

Serum cytokines were tested on a subset of pigs to determine the optimal time point for detection of INFα and γ. Optimal time point was determined by the amount of positive samples that could be detected by the assay at each time point. INFα was then measured on d 10 post-inoculation, and no effect of PRRSV or MCFA were observed (Figure 4.4). Serum INFγ concentrations were measured on d 14 post-inoculation, and no effect of PRRSV or MCFA was observed (Figure 4.5).

Lung tissue viral load is reported as $\log_{10}$ of Type II North American (P129) copies (Figure 4.6). Lung viral load was zero for both uninfected treatments at d 10 and 28 post-inoculation, which confirmed uninfected pigs remained negative throughout the challenge experiment. PRRSV concentration was ($P < 0.05$) 6.08 and 6.01 $\log_{10}$ in lung tissue of control- and MCFA-fed PRRSV infected pigs on d 10 post-inoculation, respectively. Lung tissue viral concentration at d 28 post-inoculation was 6.03 log in both control- and MCFA-fed PRRSV infected pigs. There was no difference in lung tissue viral concentration between the control- and MCFA-fed PRRSV infected pigs. There was a trend ($P < 0.10$) for control-fed PRRSV infected pig's viral load to be increased on d 10 vs d 28 post-inoculation.

Tonsil tissue viral load is reported as $\log_{10}$ of Type II North American (P129) copies (Figure 4.7). PRRSV was not detectable in tonsil tissue from uninfected treatments at d 10 and 28 post-inoculation, which confirmed uninfected pigs were negative throughout the challenge experiment. PRRSV concentration in tonsil was 3.91 and 3.56 Log_{10} on d 10 post-inoculation for control- and MCFA- fed PRRSV infected pigs, respectively. There was no difference on d 10 or 28 post-inoculation in tissue viral concentrations between control- and MCFA-fed PRRSV infected pigs.

The effect of PRRSV infection on gut morphology analysis was determined using H&E staining of jejunum and ileum sections. Measurements of villi and crypt were conducted on images and exact techniques are depicted in (Figure 4.9). Jejunum villi height and crypt depth increased in size from d 10 to 28 post-inoculation. This was expected due to this experiment being conducted in nursery pigs where the intestines should be growing, recovering, and developing. Jejunum villi height on d 10 post-inoculation was reduced ($P < 0.05$) in PRRSV infected pigs (Figure 4.10). However, there appears to be a compensatory effect by d 28 post-inoculation with the PRRSV infected pigs having increased ($P < 0.05$) villi height. Ileal villi height and crypt depth increased from d 10 to 28 post-inoculation, similar to jejunum. Ileal crypt depth at d 10 post-inoculation was reduced ($P < 0.05$) in PRRSV infected pigs (Figure 4.11). However, there appears to be a recovery by d 28 post-inoculation with PRRSV infected pigs having similar crypt depths to the uninfected pigs.

4.5   Discussion

The objective of this experiment was to evaluate the effectiveness of an MCFA combination provided in the diet of nursery pigs to inhibit or reduce PRRSV infection. The MCFA combination used was a 1:1 ratio of C8:C12 mixed in the diet to provide 0.30% total MCFA. MCFA have been reported to be effective against a wide variety of pathogens (Yoon et al., 2018) and pose a reduced risk of bacterial resistance. MCFA have been reported to disrupt the cellular membrane of bacteria and envelope viruses (Kim and Rhee 2013; Jackman et al., 2020). MCFA have immunomodulatory effects in animals, specifically in T cell lymphocytes, shifting the response to TH1 and TH17 response through the GPR84 receptor (Huang et al., 2014). Additionally MCFA have been reported to be anti-inflammatory with reduced cytokine production (Zhang et al., 2016) and improved immune response (Martínez-Vallespín et al., 2016).

The PRRSV infection model was successful in achieving a PRRSV challenge as observed in serum viral load. PRRSV infection was detected at d 3 post-inoculation and continued to increase through d 10 to 14 post-inoculation. These results agree with other studies that have reported viral peak is around 10 d post inoculation (Greiner et al., 2000; Liu et al., 2013; Rochell et al., 2015). PRRSV infection led to reduced feed intake and in turn reduced gain in this experiment which is aligned with other studies (Greiner et al., 2000; Toepfer-berg et al., 2004). In this experiment, overall ADG was reduced by 18% and feed intake was reduced 28% with PRRSV infection. This was a smaller reduction in ADG than Che et al., (2011; 59% reduction) and Liu et al., (2013; 47% reduction) reported. This could be explained in part by the lack of a strong viremic effect in this experiment (no effect of PRRSV infection on rectal temperatures), which has been reported in other work. Therefore, it is possible the PRRSV strain used in this experiment could be less virulent as well.

The impacts of PRRSV on INFγ has been inconsistent in previous research reported by others. INFγ concentrations during a PRRSV challenge range from not detectable as reported by Liu et al. (2013), to no difference measured in the present experiment, to an increased concentration reported by Toepfer-berg et al., (2004). Each of the studies measured cytokines within the range of d 0 to 14 post-inoculation, suggesting that PRRSV strain variability could be causing these irregularities.

PRRSV was recovered from tonsil tissue in this experiment at d 10 and 28 post-inoculation and is consistent with other research detecting PRRSV in the tonsil (Allende et al., 2000; Wills et al., 2003). Previous research on tonsil viral load followed the pigs for more days post-inoculation compared to the current experiment, which is limited to 28 d post-inoculation, and therefore persistence beyond d 28 cannot be evaluated. PRRSV was detected in lung tissue on both d 10

and 28 post-inoculation, which does align with other research that has reported PRRSV in lung tissue (Lamontagne et al., 2003; Li et al., 2016).

PRRSV infected pigs had reduced gut villi height in the jejunum and crypt depth in the ileum on d 10 post-inoculation. Reduced villi height decreases the surface area of intestine and leads to reduced digestive efficiency and average daily gain which could partially explain the decreased growth performance measured in this experiment. Other research with PRRSV infected pigs has reported changes in gut morphology including reduced villi height (Escabar et al., 2006). However, there is potential for reductions in villi height to be more than just an effect of the PRRSV infection alone. We have reported in this experiment as have others (Che et al., 2011 and Liu et al., 2013) that feed intake is reduced with PRRSV infection. Reduced feed intake has been reported to cause gut damage and reduce villus height in the jejunum (Habold et al., 2014). Therefore, the morphological changes during a PRRSV infection are multifaceted and not solely the direct effect of the virus.

Nutritional impacts on health require the intercommunication between gut tissue, the central nervous system, and the immune system (Rehfeld, 2004; Janssen & Depoortere, 2013). Understanding the vast interaction of the gut with other regulatory systems is still not complete but does provide targets for impacting health and performance in the animal as well as targets for therapeutic products to prevent infection and disease spread. Overall, no effects of MCFA on PRRSV viral load or performance were observed in this experiment. MCFA were effective at reducing viral replication of PRRSV in MARC-145 cells in vitro (Chapters 2 and 3). However, similar results were not observed in this experiment. In order to fully understand the application of MCFA to inhibit PRRSV infection in pigs, more studies should be conducted to evaluate the

effect of MCFA in vitro with porcine alveolar macrophages, different forms of MCFA, different

dietary inclusion levels of MCFA, and field strains of PRRSV.

4.6 Literature cited

Albina, E., C. Carrat, and B. Charley. 1998. Interferon-α response to swine arterivirus (PoAV), the porcine reproductive and respiratory syndrome virus. J. Interf. Cytokine Res. 18:485–490. doi:10.1089/jir.1998.18.485.

Allende, R., W. W. Laegreid, G. F. Kutish, J. A. Galeota, R. W. Wills, and F. A. Osorio. 2000. Porcine Reproductive and Respiratory Syndrome Virus: Description of Persistence in Individual Pigs upon Experimental Infection †. Available from: http://jvi.asm.org/

Brar, M. S., M. Shi, R. K.-H. Hui, and F. C.-C. Leung. 2014. Genomic Evolution of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Isolates Revealed by Deep Sequencing. PLoS One. 9. doi:10.1371/journal.pone.0088807.

Budden, K. F., S. L. Gellatly, D. L. A. Wood, M. A. Cooper, M. Morrison, P. Hugenholtz, and P. M. Hansbro. 2017. Emerging pathogenic links between microbiota and the gut–lung axis. Nat. Rev. Microbiol. 15:55–63. doi:10.1038/nrmicro.2016.142. Available from: http://www.nature.com/articles/nrmicro.2016.142

Calder, P. C. 2008. The relationship between the fatty acid composition of immune cells and their function. doi:10.1016/j.plefa.2008.09.016. Available from: www.elsevier.com/locate/plefa

Che, T. M., R. W. Johnson, K. W. Kelley, W. G. Van Alstine, K. A. Dawson, C. A. Moran, and J. E. Pettigrew. 2011. Mannan oligosaccharide improves immune responses and growth efficiency of nursery pigs experimentally infected with porcine reproductive and respiratory syndrome virus1,2. J. Anim. Sci. 89:2592–2602. doi:10.2527/jas.2010-3208. Available from: https://academic.oup.com/jas/article/89/8/2592-2602/4764600

Cochrane, R. A., A. R. Huss, G. C. Aldrich, C. R. Stark, and C. K. Jones. 2016. Evaluating Chemical Mitigation of Salmonella Typhimurium ATCC 14028 in Animal Feed Ingredients. J. Food Prot. 79:672–676. doi:10.4315/0362-028X.JFP-15-320. Available from: https://meridian.allenpress.com/jfp/article/79/4/672/174449/Evaluating-Chemical-Mitigation-of-Salmonella

Dierick, N., J. Decuypere, and I. Degeyter. 2003. The combined use of whole cuphea seeds containing medium chain fatty acids and an exogenous lipase in piglet nutrition. Arch. Anim. Nutr. 57:49–63. doi:10.1080/0003942031000086626. Available from: http://www.tandfonline.com/doi/abs/10.1080/0003942031000086626

Escobar, J., T. L. Toepfer-Berg, J. Chen, W. G. Van Alstine, J. M. Campbell, and R. W. Johnson. 2006. Supplementing drinking water with Solutein did not mitigate acute morbidity effects of porcine reproductive and respiratory syndrome virus in nursery pigs. J. Anim. Sci. 84. doi:10.2527/jas.2005-616.

Furness, J. B., L. R. Rivera, H. J. Cho, D. M. Bravo, and B. Callaghan. 2013. The gut as a sensory organ. Nat. Rev. Gastroenterol. Hepatol. 10:729–740. doi:10.1038/nrgastro.2013.180. Available from: https://www.nature.com/articles/nrgastro.2013.180

Ge, Y., X. Wang, Y. Guo, Junting Yan, A. Abuduwaili, K. Aximujiang, Jie Yan, and M. Wu. 2021. Gut microbiota influence tumor development and Alter interactions with the human immune system. J. Exp. Clin. Cancer Res. 40. doi:10.1186/s13046-021-01845-6.

Gebhardt, J. T., K. A. Thomson, J. C. Woodworth, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, and S. S. Dritz. 2017. Evaluation of Medium Chain Fatty Acids as a Dietary Additive in Nursery Pig Diets. Kansas Agric. Exp. Stn. Res. Reports. 3. doi:10.4148/2378-5977.7463. Available from: https://newprairiepress.org/kaesrr/vol3/iss7/10

Greiner, L. L., T. S. Stahly, and T. J. Stabel. 2000. Quantitative relationship of systemic virus concentration on growth and immune response in pigs. J. Anim. Sci. 78:2690. doi:10.2527/2000.78102690x. Available from: https://academic.oup.com/jas/article/78/10/2690-2695/4670875

Habold, C., C. Chevalier, S. Dunel-Erb, C. Foltzer-Jourdainne, Y. Le Maho, and J. -H. Lignot. 2004. Effects of fasting and refeeding on jejunal morphology and cellular activity in rats in relation to depletion of body stores. Scand. J. Gastroenterol. 39. doi:10.1080/00365520410004514.

Holtkamp, D. J., J. B. Kliebenstein, E. J. Neumann, J. J. Zimmerman, H. F. Rotto, T. K. Yoder, C. Wang, P. E. Yeske, C. L. Mowrer, and C. A. Haley. 2013. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. Available from: http://www.aasv.org/shap.html.

Huang, Q., D. Feng, K. Liu, P. Wang, H. Xiao, Y. Wang, S. Zhang, and Z. Liu. 2014. A medium-chain fatty acid receptor Gpr84 in zebrafish: Expression pattern and roles in immune regulation. Dev. Comp. Immunol. 45:252–258. doi:10.1016/j.dci.2014.03.017. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0145305X14000858

Jackman, J. A., R. D. Boyd, and C. C. Elrod. 2020. Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation. J. Anim. Sci. Biotechnol. 11:44. doi:10.1186/s40104-020-00446-1. Available from: https://jasbsci.biomedcentral.com/articles/10.1186/s40104-020-00446-1

Janssen, S., and I. Depoortere. 2013. Nutrient sensing in the gut: new roads to therapeutics? Trends Endocrinol. Metab. 24:92–100. doi:10.1016/j.tem.2012.11.006. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1043276012002160

Kim, S. A., and M. S. Rhee. 2013. Marked synergistic bactericidal effects and mode of action of medium-chain fatty acids in combination with organic acids against Escherichia coli O157: H7. Appl. Environ. Microbiol. 79:6552–6560. doi:10.1128/AEM.02164-13.

Lamontagne, L., C. Pagé, R. Larochelle, and R. Magar. 2003. Porcine Reproductive and Respiratory Syndrome Virus Persistence in Blood, Spleen, Lymph Nodes, and Tonsils of Experimentally Infected Pigs Depends on the Level of CD8 high T Cells. Viral Immunol. 16:395–406. doi:10.1089/088282403322396181. Available from: https://www.liebertpub.com/doi/10.1089/088282403322396181

Lerner, A. B., R. A. Cochrane, J. T. Gebhardt, S. S. Dritz, C. K. Jones, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. Bai, E. Porter, J. Anderson, P. C. Gauger, D. R. Magstadt, J. Zhang, B. Bass, T. Karnezos, B. de Rodas, and J. C. Woodworth. 2020. Effects of medium chain fatty acids as a mitigation or prevention strategy against porcine epidemic diarrhea virus in swine feed. J. Anim. Sci. 98. doi:10.1093/jas/skaa159. Available from: https://academic.oup.com/jas/article/doi/10.1093/jas/skaa159/5843591

Li, Z., Y. He, X. Xu, X. Leng, S. Li, Y. Wen, F. Wang, M. Xia, S. Cheng, and H. Wu. 2016. Pathological and immunological characteristics of piglets infected experimentally with a HP-PRRSV TJ strain. doi:10.1186/s12917-016-0854-x.

Liu, Y., T. M. Che, M. Song, J. J. Lee, J. A. S. Almeida, D. Bravo, W. G. Van Alstine, and J. E. Pettigrew. 2013. Dietary plant extracts improve immune responses and growth efficiency of pigs experimentally infected with porcine reproductive and respiratory syndrome virus1. J. Anim. Sci. 91:5668–5679. doi:10.2527/jas.2013-6495. Available from: https://academic.oup.com/jas/article/91/12/5668/4717124

Martínez-Vallespín, B., W. Vahjen, and J. Zentek. 2016. Effects of medium-chain fatty acids on the structure and immune response of IPEC-J2 cells. Cytotechnology. 68:1925–1936. doi:10.1007/s10616-016-0003-1. Available from: http://link.springer.com/10.1007/s10616-016-0003-1

Niederwerder, M. C., S. Dee, D. G. Diel, A. M. M. Stoian, L. A. Constance, M. Olcha, V. Petrovan, G. Patterson, A. G. Cino-Ozuna, and R. R. R. Rowland. 2020. Mitigating the risk of African swine fever virus in feed with anti-viral chemical additives. Transbound. Emerg. Dis. doi:10.1111/tbed.13699

Rehfeld, J. F. 2004. A Centenary of Gastrointestinal Endocrinology. Horm. Metab. Res. 36:735–741. doi:10.1055/s-2004-826154. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-2004-826154

Rochell, S. J., L. S. Alexander, G. C. Rocha, W. G. Van Alstine, R. D. Boyd, J. E. Pettigrew, and R. N. Dilger. 2015. Effects of dietary soybean meal concentration on growth and immune response of pigs infected with porcine reproductive and respiratory syndrome virus. J. Anim. Sci. 93:2987–2997. doi:10.2527/jas.2014-8462.

Toepfer-Berg, T. L., J. Escobar, W. G. Van Alstine, D. H. Baker, J. Salak-Johnson, and R. W. Johnson. 2004. Vitamin E supplementation does not mitigate the acute morbidity effects of porcine reproductive and respiratory syndrome virus in nursery pigs1. J. Anim. Sci. 82:1942–1951. doi:10.2527/2004.8271942x. Available from: https://academic.oup.com/jas/article/82/7/1942/4790469

Wills, R. W., A. R. Doster, J. A. Galeota, J.-H. Sur, and F. A. Osorio. 2003. Duration of Infection and Proportion of Pigs Persistently Infected with Porcine Reproductive and Respiratory Syndrome Virus †. J. Clin. Microbiol. 41:58–62. doi:10.1128/JCM.41.1.58-62.2003. Available from: http://jcm.asm.org/

Xiao, C., J. Hsieh, K. Adeli, and G. F. Lewis. 2011. Gut-liver interaction in triglyceride-rich lipoprotein metabolism. Am. J. Physiol. Metab. 301:E429–E446. doi:10.1152/ajpendo.00178.2011. Available from: https://www.physiology.org/doi/10.1152/ajpendo.00178.2011

Yoon, B., J. Jackman, E. Valle-González, and N.-J. Cho. 2018. Antibacterial Free Fatty Acids and Monoglycerides: Biological Activities, Experimental Testing, and Therapeutic Applications. Int. J. Mol. Sci. 19:1114. doi:10.3390/ijms19041114. Available from: www.mdpi.com/journal/ijms

Zentek, J., S. Buchheit-Renko, K. Männer, R. Pieper, and W. Vahjen. 2012. Intestinal concentrations of free and encapsulated dietary medium-chain fatty acids and effects on gastric microbial ecology and bacterial metabolic products in the digestive tract of piglets. Arch. Anim. Nutr. 66:14–26. doi:10.1080/1745039X.2011.644916. Available from: http://www.tandfonline.com/doi/abs/10.1080/1745039X.2011.644916

Zhang, M. S., A. Sandouk, and J. C. D. Houtman. 2016. Glycerol Monolaurate (GML) inhibits human T cell signaling and function by disrupting lipid dynamics. Sci. Rep. 6:30225. doi:10.1038/srep30225. Available from: http://www.nature.com/articles/srep30225

Table 4.1. Composition of Nursery Diets, as fed.

	Phase 1 1.13 kg/pig	Phase 2 2.72 kg/pig	Phase 3 6.35 kg/pig	Phase 4 to end of trial
Ingredient, %				
Corn, ground	36.41	46.77	53.57	61.305
Soybean meal	14.14	17.50	25.00	31.50
Choice White Grease	0	0	0	2.70
Soybean oil	4.70	3.70	2.70	0
Limestone	0.81	0.91	0.87	1.29
Monocalcium phosphate	0.30	0.46	0.55	0.82
Vitamin Premix[1]	0.25	0.25	0.25	0.25
Trace Mineral Premix[2]	0.125	0.125	0.125	0.125
Selenium Premix[3]	0.05	0.05	0.05	0.05
Phytase[4]	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.30	0.35
Plasma Protein	5.00	2.50	0	0
Blood Meal	1.50	1.00	0	0
Soy Concentrate	5.00	3.50	2.25	0
Fish Meal	4.00	4.00	4.00	0
Dried Whey	25.75	17.15	8.60	0
L-Lysine HCL	0.15	0.28	0.31	0.38
DL-methionine	0.23	0.23	0.185	0.17
L-Threonine	0.06	0.12	0.125	0.14
L-Tryptophan	0.01	0.025	0.02	0.01
Copper Sulphate	0	0	0	0.10
Zinc Oxide	0.375	0.35	0.28	0
Hemicell Enzyme[5]	0.04	0.04	0.04	0.04
Clarify Larvicide[6]	0.14	0.09	0.08	0.07
MCFA Premix [7]/Soy oil for control	0.60	0.60	0.60	0.60
Calculated Chemical Composition				
ME, Kcal/kg	3591	3543	3492	3467
SID Lys, %	1.55	1.45	1.35	1.25
Ca, %	0.85	0.85	0.80	0.75
Phos, %	0.70	0.66	0.64	0.56
Av.P, %	0.55	0.50	0.45	0.35
Lactose, %	18.00	12.00	6.00	0

[1]Vitamin premix provided per kg of the diet: vitamin A, 6615 IU; vitamin D3, 662 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acids, 22 mg; niacin, 33 mg; vitamin B_{12}, 38.6 mg

[2]Trace mineral premix provided per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15 mg; copper, 11.3 mg; iodine, 0.46 mg

[3]Selenium premix provided selenium at 0.3 ppm

[4]Phytase provided at 600 FTU

[5]Hemicell is a β mananase enzyme

[6]Clarifly (Central Life Sciences, Schaumburg, IL) diflubenuron: phase 1, 9.4 ppm; phase 2, 6 ppm; phase 3, 5.4 ppm; phase 4, 4.7 ppm

[7]Medium Chain Fatty Acid (MCFA) provided 0.15% C8; 0.15% C12

Table 4.2. Composition of Nursery Diets, as fed.

	Phase 3 6.35 kg/pig	Phase 4 to end of trial
Analyzed Chemical Composition %		
Crude Protein	21.06	19.4
Crude Fat	4.49	4.46
Crude Fiber	2.5	2.65
C8	0.084	0.06
C12	0.123	0.09

[1]Vitamin premix provided per kg of the diet: vitamin A, 6615 IU; vitamin D3, 662 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acids, 22 mg; niacin, 33 mg; vitamin B_{12}, 38.6 mg

[2]Trace mineral premix provided per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15 mg; copper, 11.3 mg; iodine, 0.46 mg

[3]Selenium premix provided selenium at 0.3 ppm

[4]Phytase provided at 600 FTU

[5]Hemicell is a β mananase enzyme

[6]Clarifly (Central Life Sciences, Schaumburg, IL) diflubenuron: phase 1, 9.4 ppm; phase 2, 6 ppm; phase 3, 5.4 ppm; phase 4, 4.7 ppm

[7]Medium Chain Fatty Acid (MCFA) provided 0.15% C8; 0.15% C12

Table 4.3. Primers used for quantitative polymerase chain reaction

Primer	Sequence
PRRSV – P 129	
P129 – Forward	5'-ACCAGCCATTTCCTTGACAC-3'
P129 – Reverse	5'-CGCAAGCCTAATAACGAAGC-3'
Housekeeping	
RPL19 – Forward	5'-AACTCCCGTCAGCAGATCC-3'
RPL19 - Reverse	5'-AGTACCCTTCCGCTTACCG-3'

Table 4.4. Effect of dietary treatment and PRRSV[1] infection on nursery body weights (kg)[2].

| | Dietary Treatments | | | | | P- value | | |
| | Control | | MCFA[3] | | | | | |
Day post-inoculation	Non-infected	PRRSV	Non-infected	PRRSV	SEM	*PRRSV*	*MCFA*	*PRRSV*MCFA*
-5	7.50	7.52	7.50	7.49	0.68	0.951	0.563	0.691
0	8.59	8.64	8.57	8.42	0.75	0.658	0.266	0.360
3	9.53	9.52	9.44	9.18	0.84	0.585	0.393	0.607
7	11.02	10.85	10.64	10.49	0.93	0.606	0.259	0.965
10	12.12	11.71	11.97	11.47	1.01	0.103	0.449	0.852
14	14.37	13.37	13.82	12.83	1.14	0.015	0.137	0.991
21	18.70	16.47	17.89	15.89	1.40	<0.001	0.127	0.795
28	22.83	20.24	22.39	19.78	1.61	0.002	0.483	0.994

[1]Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type II North American (P129)
[2]Data are means of 4 pens/treatment with of 6-7 pigs per pen fed experimental diets for a period of 33 days from -5 to 28 d post-inoculation
[3]Medium Chain Fatty Acids (MCFA) C8:C12 in a 1:1 ratio (wt:wt) fed at 0.30% total MCFA.

Table 4.5. Effect of dietary treatment and PPRSV[1] infection on nursery growth performance[2].

| | Dietary Treatments | | | | | P-value | | |
| | Control | | MCFA[2] | | | | | |
Performance	Uninfected	PRRSV	Uninfected	PRRSV	SEM	*PRRS*	*MCFA*	*PRRSV*MCFA*
Days post-inoculation								
d 0-3								
ADG, kg	0.31	0.29	0.29	0.25	0.05	0.588	0.546	0.856
ADFI, kg	0.43	0.53	0.56	0.53	0.09	0.668	0.364	0.379
G:F	0.75	0.58	0.53	0.58	0.14	0.645	0.398	0.394
d 3-7								
ADG, kg	0.37	0.34	0.29	0.33	0.04	0.999	0.194	0.320
ADFI, kg	0.61	0.37	0.45	0.31	0.05	0.002	0.033	0.342
G:F	0.61	0.95	0.65	1.12	0.11	0.004	0.342	0.575
d 7-10								
ADG, kg	0.36	0.28	0.44	0.34	0.05	0.071	0.159	0.804
ADFI, kg	1.00	1.01	1.09	0.56	0.11	0.010	0.049	0.008
G:F	0.39	0.28	0.41	0.63	0.06	0.372	0.011	0.016
d 10-14								
ADG, kg	0.57	0.40	0.44	0.33	0.06	0.015	0.072	0.563
ADFI, kg	0.76	0.26	0.43	0.33	0.08	0.005	0.140	0.036
G:F	0.79	1.99	1.17	1.02	0.33	0.129	0.366	0.059
d 14-21								
ADG, kg	0.61	0.44	0.58	0.44	0.04	0.001	0.619	0.619
ADFI, kg	0.96	0.68	0.92	0.63	0.09	0.002	0.512	0.999
G:F	0.65	0.70	0.65	0.70	0.09	0.518	0.958	0.980
d 21-28								
ADG. kg	0.69	0.63	0.75	0.65	0.05	0.077	0.326	0.608
ADFI, kg	1.34	1.04	1.25	0.73	0.08	<0.001	0.034	0.178
G:F	0.52	0.61	0.60	0.88	0.04	0.001	0.001	0.018
d 0-28								
ADG, kg	0.52	0.42	0.50	0.41	0.03	0.001	0.510	0.832
ADFI, kg	0.89	0.67	0.82	0.53	0.05	<0.001	0.015	0.378
G:F	0.58	0.63	0.61	0.78	0.04	0.006	0.015	0.088

[1]Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type II North American (P129)
[2]Data are means of 4 pens/treatment with of 6-7 pigs per pen fed experimental diets for a period of 33 days from -5 to 28 d post-inoculation
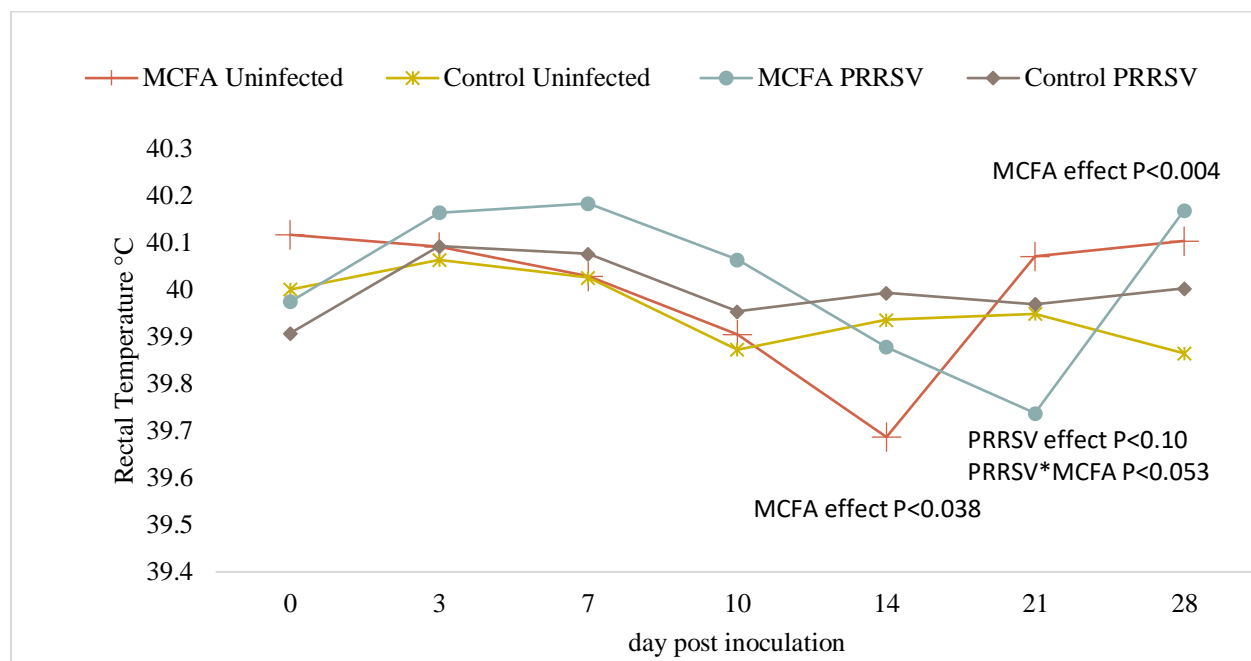[3]Medium Chain Fatty Acids (MCFA) C8:C12 in a 1:1 ratio (wt:wt) fed at 0.30% total MCFA.

Figure 4.1. Effect of Dietary treatment and PRRSV infection on rectal body temperatures of pigs. D 14 post-inoculation there was an effect of MCFA (P<0.05) MCFA-fed uninfected pigs had reduced rectal temperatures when compared to control fed uninfected and PRRSV infected pigs with the MCFA-fed PRRSV pigs being intermediate. MCFA-fed PRRSV pigs tended (P <0.10) to have reduced rectal temperatures at d 21 post-inoculation as an efedct of PRRSV when compared to both control-fed and MCFA-fed uninfected pigs with control-fed PRRSV infected pigs being intermediate. There was an interaction of PPRSV*MCFA with MCFA fed PRRSV infected pigs having a lower temperature and control fed PRRSV pigs having a higher temperature when compared to the same diets uninfected pigs. D 28 post-inoculation there was an MCFA effect (P<0.05) with MCFA-fed uninfected and PRRSV infected pigs had increased rectal temperatures when compared to the control-fed uninfected pigs with control-fed PRRSV infected pigs intermediate.
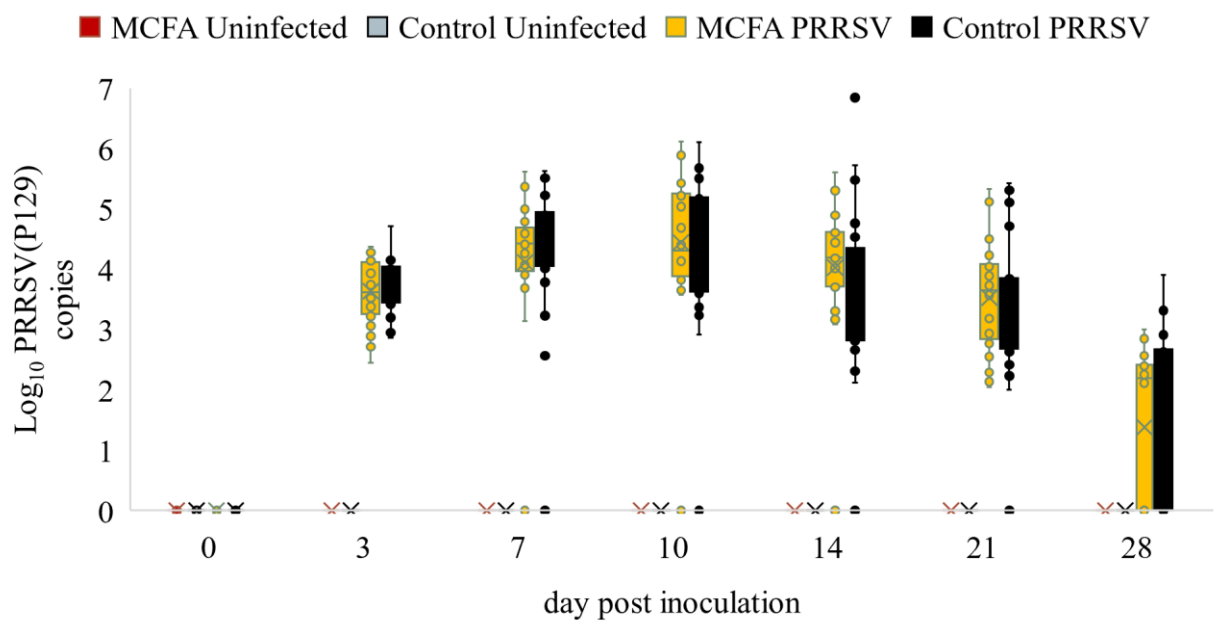
Figure 4.2. The effect of dietary treatment and PRRSV infection on serum viral load concentration. Uninfected pigs were negative for PRRSV in serum at all time points. There was no difference in PRRSV serum viral load between PRRSV infected pigs.
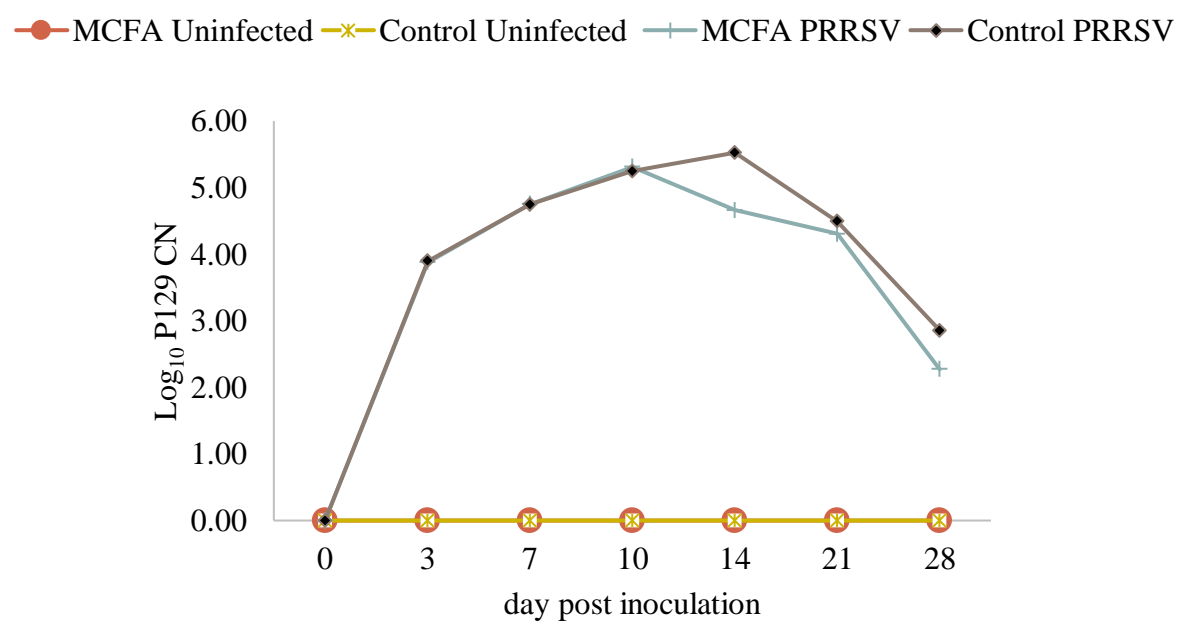
Figure 4.3. Effect of dietary treatment and PRRSV infection on serum viral load area under the curve analysis. There was no difference in area under the curve for PRRSV serum levels between treatments.
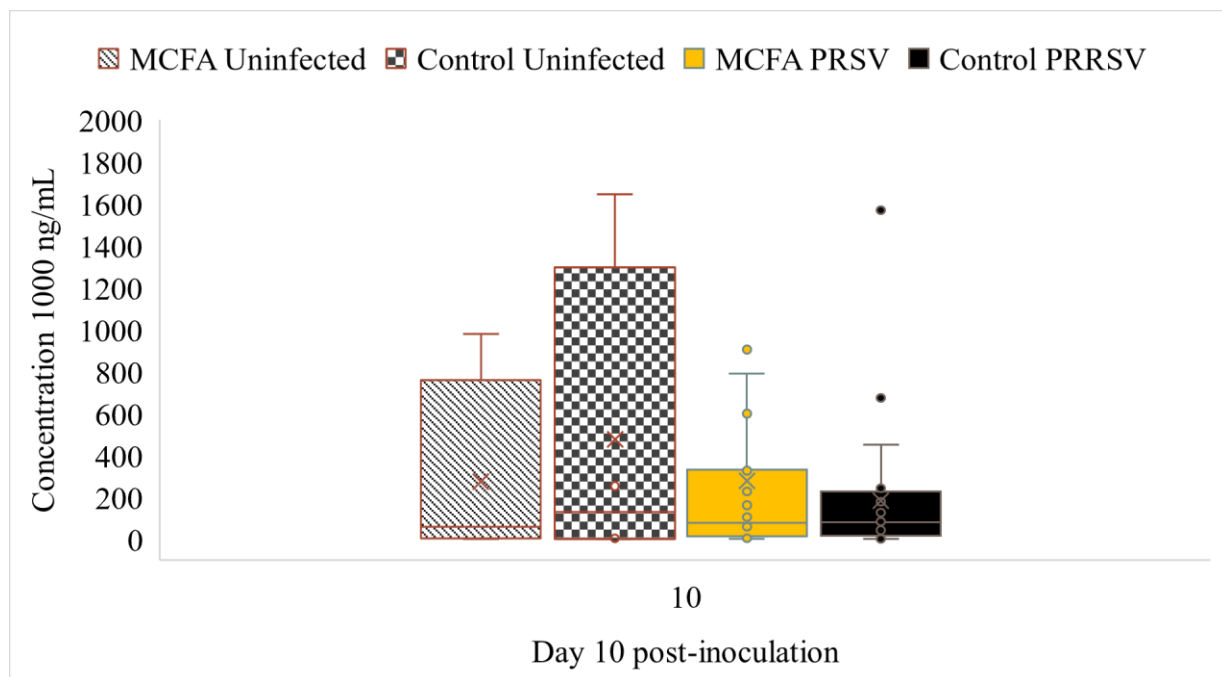
Figure 4.4. Effect of dietary treatment and PRRSV infection on serum IFNα concentraiton. There was no difference in IFNα concentration among treatments.
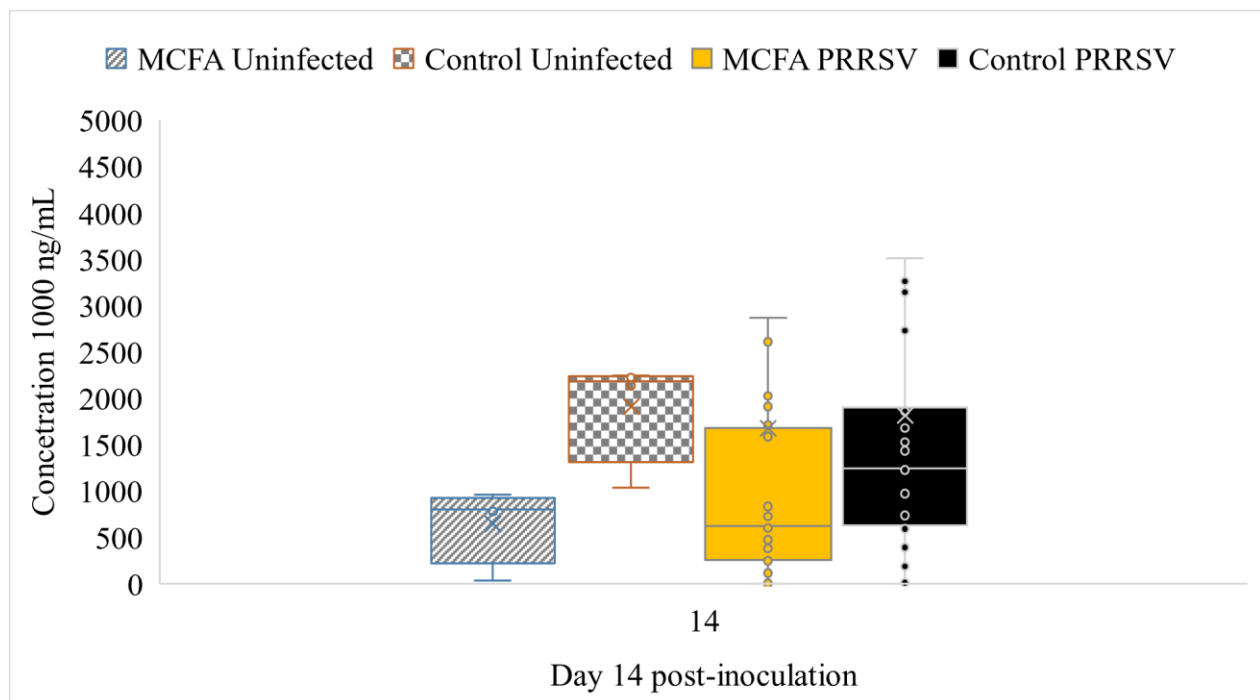
Figure 4.5. Effect of dietary treatment and PRRSV infection on serum IFNγ concentraiton. There was no differnce in IFNγ concnetrations among treatments.
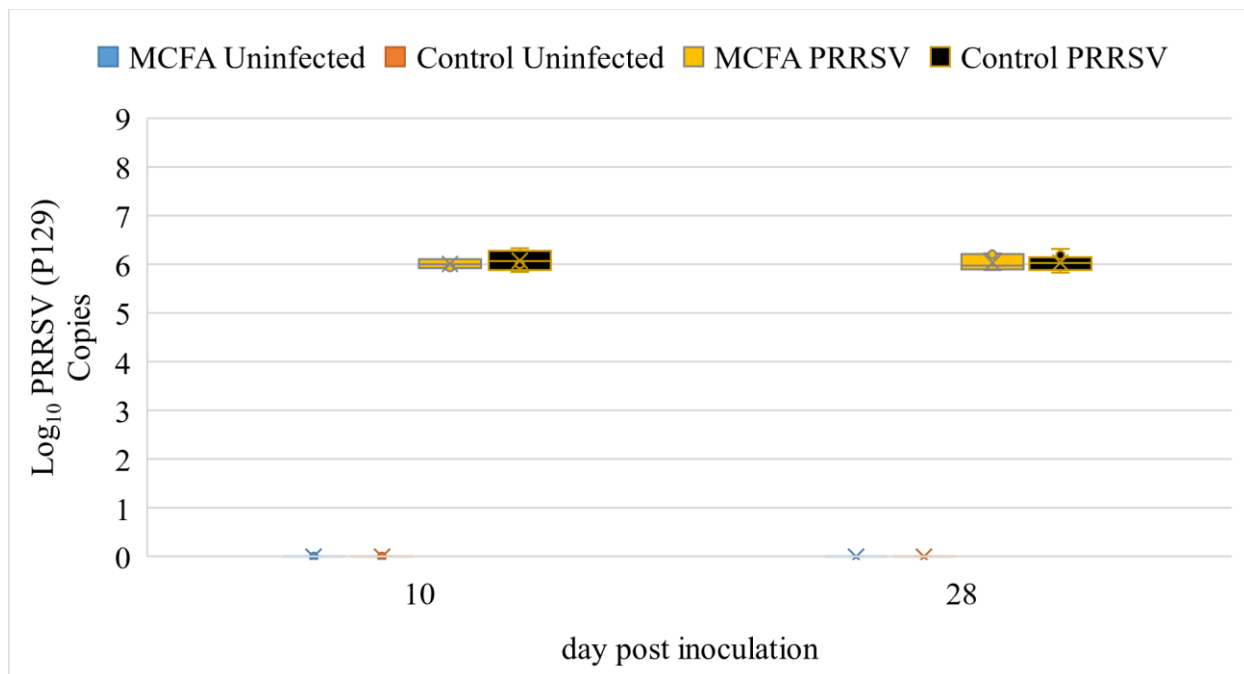
Figure 4.6. The effect of dietary treatment and PRRSV infection on lung tissue viral load concentration. PRRSV infection was not detected in the unifected pigs at d 10 or 28 post-inoculation. PRRSV infection was detected in lung tissue at both d 10 and 28 post-inoculation and there was no difference between PRRSV infected pigs.
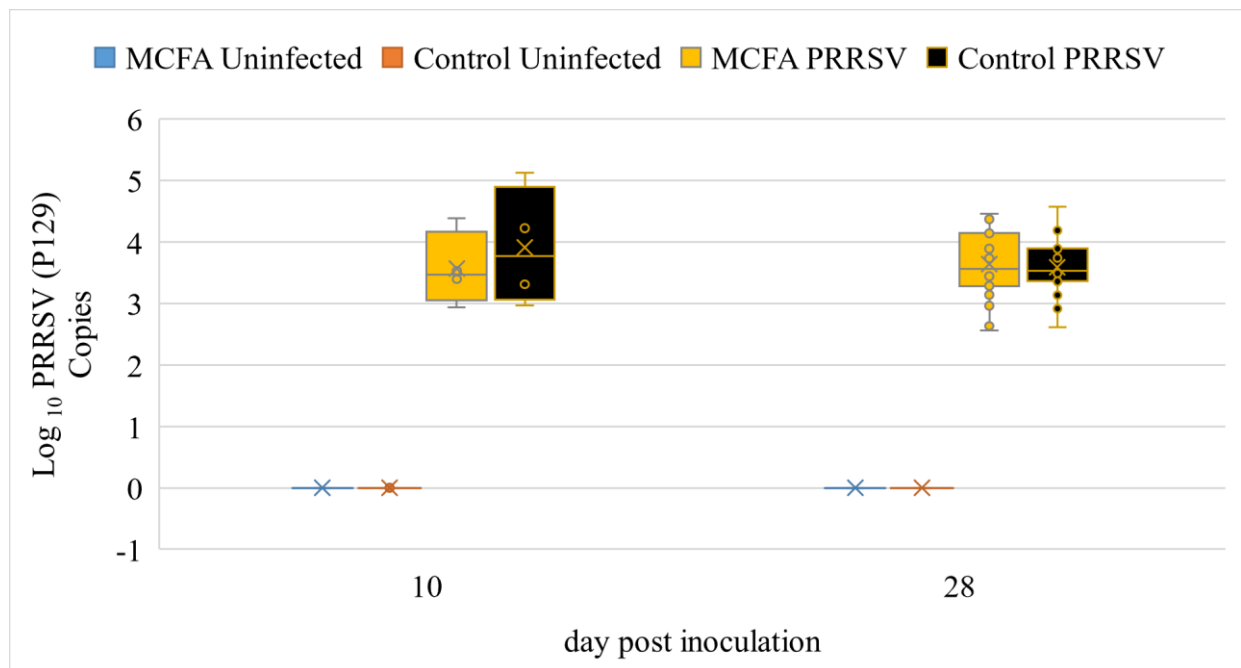
Figure 4.7. The effect of dietary treatment and PRRSV infection on tonsil tissue viral load concentrations. PRRSV infection was not detected in the unifected pigs at d 10 or 28 post-incoulation. PRRSV was detected in tonsil tissue at both d10 and 28 post-inoculation for challenged pigs. There was no differnece between the PRRSV infected pigs tonsil viral load concentration.
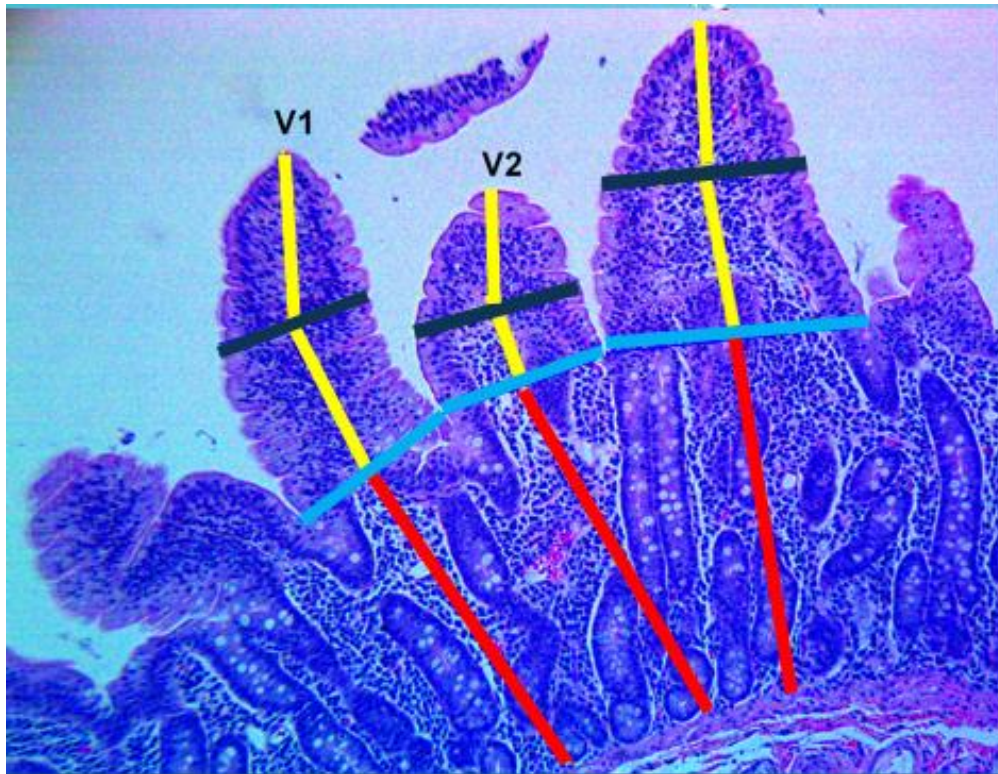
Figure 4.8. Measurements used for gut morphology analysis. The yellow bar depicts how villi height was determined. The red bar is indicative of crypt depth measurement area. The black cross bar is where the mid width measurement was taken. Base width was measured using the blue cross bar alignment

Figure 4.9. The effect of PRRSV infection on jejunum gut morphology. PRRSV infection reduced jejunal villi height (P < 0.05) at d 10 post-inoculation and increased (P< 0.05) jejunal villi height at d 28 post-inoculation. No difference was detected in crypt depth at d 10 or 28 post-inoculation
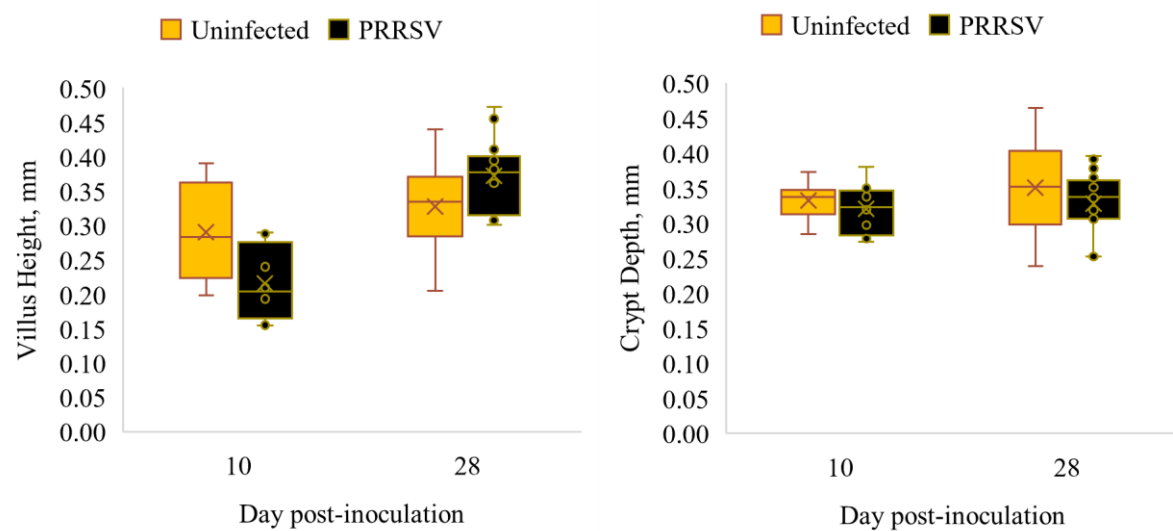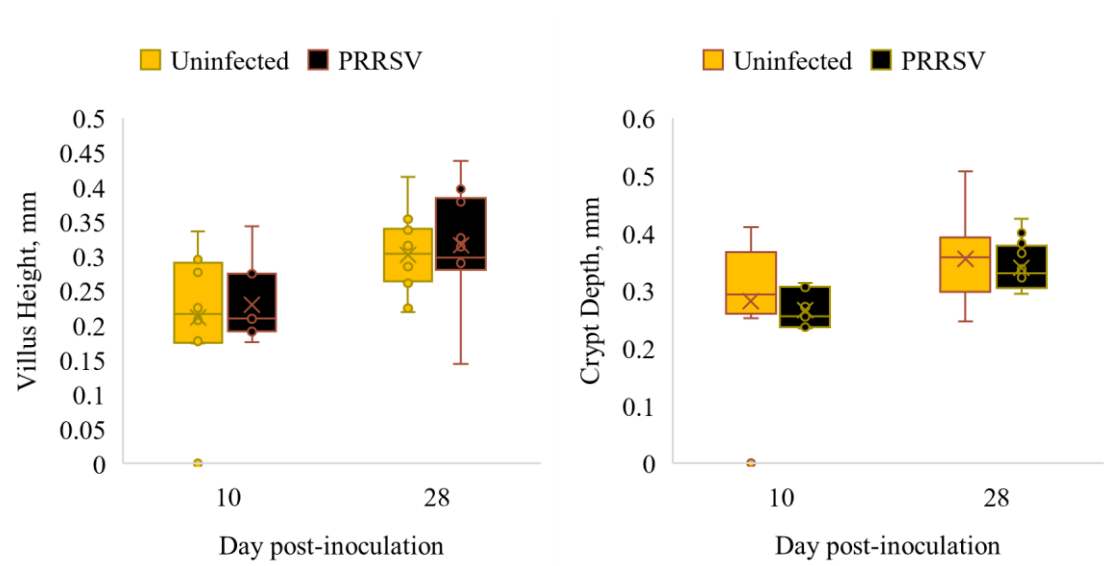
Figure 4.10. The effect of PRRSV infection on ileum gut morphology. PRRSV infection reduced ileal crypt depth (P < 0.05) at d 10 post-inoculation and at d 28 post-inoculation there was no difference. No difference was detected in villus height at d 10 or 28 post-inoculation.

CHAPTER 5.   SUMMARY

In summary, understanding that PRRSV infection leads to increased inflammation and is immunosuppressive provides the basis for providing MCFA through the diet to support immune function. MCFA are immunomodulatory and impact T cell signaling through GPR84 and monocyte differentiation through GPR40. MCFA C6, C8, C10, C12 have been reported to polarize naïve T cells to a Th17 and Th1 phenotype and influence neutrophils through the activation of GPR84. The research presented earlier on feed mitigation using MCFA demonstrates that MCFA can inhibit viral infection in in-vivo studies. These findings suggest that it is possible to influence the immune response through nutrition and leads to the working hypothesis that dietary components could be capable of impacting the immune response to a respiratory disease infection.

The objective of the research undertaken as part of this dissertation was first to determine the impact of MCFA on viral replication of PRRSV. Chapter 2 evaluated the effect of individual MCFA on PRRSV replication in vitro using MARC-145 cells. Chapter 3 expanded on the knowledge gained in Chapter 2 and evaluated the effect of MCFA combinations on PRRSV replication in MARC-145 cells. Chapter 4 evaluated the effect of a combination of MCFA delivered through feed in a PRRSV challenge study in swine.

Chapter 2 focused on the impact of individual MCFA and their antiviral activity against PRRSV in cell culture. The results of this experiment supported our hypothesis that MCFA can reduce viral replication of PRRSV in MARC-145 cells. Results demonstrated that as MCFA chain length increased, inhibitory dose decreased. One effect of note was the effect at the highest concentration of 1000 µg/mL of MCFA (C10 and C12) where MARC-145 cells were lifted from the microtiter plates. This did not allow for evaluation of the effectiveness of MCFA at higher

concentrations. In conclusion, C8, C10, and C12 were effective at inhibiting PRRSV replication in MARC-145 cells.

Chapter 3 evaluated the efficacy of MCFA combinations (C8, C10, and C12) to reduce PRRSV replication in MARC-145 cells. The mode of action for MCFA has been speculated to be a direct effect on envelope viruses and bacteria via disruption of the viral envelope and cellular membrane integrity. Therefore, MCFA were incubated with the MARC-145 cells, the virus, or both to determine the mode of action. The results of these experiments confirmed our previous results and demonstrated that MCFA combinations reduced viral replication in both Type II and Type I PRRSV strains with MARC-145 cells. The mode of action of MCFA have been reported to effect bacterial membranes or viral envelopes. However, the results of this experiment did not demonstrate a direct effect on the PRRSV.

Chapter 4 was designed to test an MCFA combination (C8:C12) in a PRRSV challenge experiment. Overall, no effects of MCFA on PRRSV viral load or performance were observed in this experiment. MCFA were effective at reducing viral replication of PRRSV in MARC-145 cells in vitro (Chapters 2 and 3). However, similar results were not observed in this experiment.

In order to fully understand the application of MCFA to inhibit PRRSV infection in pigs, more studies should be conducted to evaluate the effect of MCFA in vitro with porcine alveolar macrophages, different forms of MCFA, different dietary inclusion levels of MCFA, and field strains of PRRSV. Next steps should be focused on issues raised from the current research in this dissertation. MARC-145 cells were removed from plates using the highest concentration of C10 and C12. Cytotoxic effects at the highest concentration was tested in a preliminary analysis and were determined to be non-cytotoxic. However, during the actual experiment, we were not able to maintain the monolayer of MARC-145 cells to fully evaluate MCFA efficacy. In future

experiments, techniques should be used to determine that cells are viable and actively dividing and that the MCFA did not cause the cells to acquiesce or go dormant. Flow cytometry analysis would be a suggestion to use in future studies to expand the potential of the cell culture experiments and gather more data points, which was limited in our current design. This would also expand our abilities to better elucidate the mode of action. Results should be confirmed in the species and cell type that is the natural host for the virus whenever possible. Therefore, next steps should confirm the MARC-145 results in porcine alveolar macrophages. Previous studies have reported that PRRSV can become attenuated when propagated in MARC-145 cells. This is of concern as we tested Type II North American (P129) and Type I European (Lelystad) PRRSV strains and both were propagated in MARC-145 cells. Our results demonstrate that pigs were successfully infected. However, the attenuation could cause reduced pathogenicity and virulence, which could partially explain some of the differences in our in vivo experiment. One main symptom of viremia in pigs infected with PRRSV is elevated rectal temperatures, and in Chapter 4, we reported no difference in rectal temperatures between PRRSV infected and uninfected pigs, even though elevated temperatures have previously been reported with Type II (P129) PRRSV infected pigs (Greiner et al., 2000). This lack of response in temperature changes could be due to attenuation of the PRRSV or that the trial was conducted in July - August in Illinois with higher environmental temperatures. Future experiments should evaluate the passage of virus used in viral stocks for inoculation, and potentially passage the PRRSV through the animal target species prior to the challenge to confirm that pathogenicity of the virus has not changed. Another consideration for future research might be to use an active field strain of PRRSV. Due to genetic diversity and inadequate cross protection produced through vaccines today, using a field isolate for testing could help to address issues producers are facing currently.

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MCFA are found in different forms including glycerides, salts, and free fatty acids. The form of MCFA influences the function as well as the inclusion needed in feed to see a response. The largest structure/form is a glyceride where the MCFA is bound to a glycerol backbone found in triglyceride, diglyceride, or monoglyceride structural configurations. The MCFA bound to glycerol must be cleaved by lipase activity in the digestive tract. MCFA used in animal feed formulations as triglycerides are fed at higher rates to provide free fatty acids compared to directly using free fatty acids in diet formulations. MCFA used in animal feeds can also be in the salt form bound to a calcium or sodium molecule. The salt form is dissociated in the acidic environment of the stomach releasing the free MCFA, which lowers the inclusion level to provide free fatty acids. However, if your target site of action is farther into the small intestines, the salt form would be limited in providing the amount of free MCFA needed to measure a response. Free MCFA can be used in animal feed and do not require lipase activity or an acidic environment to be functional, but would have a similar caution to the salt form in terms of consideration should be given to the goal or function targeted by the use of MCFA. For an example, if you are targeting feed mitigation to reduce viral transmission you want the MCFA to be active against the pathogen in the feed immediately. Therefore, you would not use a MCFA triglyceride as they are too large to interact with pathogen membranes, making the free MCFA as well as monoglycerides a better option. Another consideration is digestion and the challenges it poses in the delivery of MCFA to the active site. MCFA are readily absorbed by the enterocytes as an energy source and can be used as cellular membrane components. They are transported via the portal vein directly to the liver for metabolism and not through micelle formation and the lymph system. Therefore, if you need the MCFA to travel farther down the intestinal tract, you would want to evaluate glyceride forms or encapsulated free MCFA.

Even with the challenges outlined, MCFA are still viable candidates for nutritional applications to improve animal health. MCFA have been reported to inhibit pathogenic bacteria and support the growth of beneficial bacteria, enhancing digestion and nutrient utilization along with promoting intestinal function and integrity. Immune function is directly impacted by the immunomodulatory effects of MCFA via T cell lymphocyte cell signaling and improved immune responses. The improvement in immune response has a direct impact on animal performance due to the energy expensive nature of immune functions. Free fatty acids interact with immune and nonimmune cells through G protein coupled receptors (GPCR) along the intestine (J. Wang et al., 2006).

Swine feed has come under heavy scrutiny in the last few years following Porcine Epidemic Diarrhea Virus (PEDV) outbreaks in 2013-2014. Ingredient sourcing and the potential for contamination coming through global trade has raised alarms about the introduction of a foreign animal disease. Treating feed with MCFA has been reported to prevent PEDV and PRRSV infection in a bioassay. Further studies have been conducted to confirm that MCFA can reduce viral transmission of PEDV. PRRSV infection has not been thought of as a feed transmission issue so this is a consideration to keep in mind. The hypothesis of how MCFA reduces viruses in feed has focused on the interaction of MCFA and the viral envelope, and our results from Chapter 3 did not confirm a direct effect on the PRRSV.

The data around the use of MCFA as a feed mitigation strategy against PEDV has led to evaluating their use with other envelope viruses including PRRSV and African Swine Fever Virus (ASFV). Treating ingredients before or after viral inoculation with ASFV resulted no functional virus detected using virus isolation (Niederwerder et al., 2020). Treating complete swine feed with MCFA has also been reported to reduce the viral transmission of PEDV and PRRSV through

feed, as demonstrated utilizing an ice block method of inoculating feed in the feed bin to simulate using feed as the vector for viral transmission.

In conclusion, nutrition impacts health and requires intercommunication between gut tissue, the central nervous system, and the immune system. Understanding of the vast interactions of the gut with other regulatory systems does provide targets for impacting health and performance in the animal as well as targets for therapeutic products to prevent infection and disease spread. This interaction of Health and Nutrition has always been in the background of performance improvements with nutritional programs. It will however continue to grow and become one of the new frontiers in animal sciences and require collaboration of nutritionists and veterinarians.

5.1   Literature Cited

Greiner, L. L., T. S. Stahly, and T. J. Stabel. 2000. Quantitative relationship of systemic virus concentration on growth and immune response in pigs. J. Anim. Sci. 78:2690. doi:10.2527/2000.78102690x. Available from: https://academic.oup.com/jas/article/78/10/2690-2695/4670875

Niederwerder, M. C., S. Dee, D. G. Diel, A. M. M. Stoian, L. A. Constance, M. Olcha, V. Petrovan, G. Patterson, A. G. Cino-Ozuna, and R. R. R. Rowland. 2020. Mitigating the risk of African swine fever virus in feed with anti-viral chemical additives. Transbound. Emerg. Dis. doi:10.1111/tbed.13699