8-2009

Effects of Syndyphalin-33 on appetite, endocrine, and immune parameters in the recently weaned pig

Sarah Jo Jenkins
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Sarah Jo Jenkins entitled "Effects of Syndyphalin-33 on appetite, endocrine, and immune parameters in the recently weaned pig." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Cheryl Kojima, Major Professor

We have read this thesis and recommend its acceptance:

Henry Kattesh, Alan Mathew

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Carolyn R. Hodges

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Effects of Syndyphalin-33 on appetite, endocrine, and immune parameters in the recently-weaned pig

A Thesis Presented for
the Master of Science Degree
The University of Tennessee, Knoxville

Sarah Jo Jenkins
August 2009

Acknowledgements

I want to thank all of those who have helped me complete my Master of Science degree in Animal Science. I would like to first and foremost thank my mentor, Dr. Cheryl Kojima, for her expert guidance in teaching me all about agriculture, swine production, and research in this field. I would like to thank Dr. Henry Kattesh for his valuable input and kind efforts into my work, and for inspiring me to become a teacher. I would also like to thank the department head, Dr. Alan Mathew, for serving on my committee and giving me this great opportunity. I am grateful to Mary Roberts for her help with my lab work, as well as the entire JARTU staff: Mark, Roger, Tammy, and Ken, for all of their help in the preparation of my project. Finally, to my loving family, friends and fiancé, thank you for always believing in me. It is your encouragement and inspiration that has driven me to reach higher and achieve my goals.

Abstract

This thesis discusses the background information regarding the physiological effects the pig encounters during weaning as well as a potential factor that can be used to assist the pig during this time. Specifically, the research focus is to assess the ability of the tri-peptide opioid agonist, Syndyphalin-33 (SD-33), to increase feed intake and body weight and modulate immune responses during the post-weaning period. The results of this research have demonstrated that SD-33 increases feed intake, transiently increases growth hormone and cortisol levels, and increases total white blood cell counts while selectively increasing monocyte numbers in healthy weaned pigs. This research also demonstrates that, although co-treatment with SD-33 during an immune challenge of *Salmonella enterica* did not result in increased feed intake, SD-33 exerted effects relating to increasing the circulating populations of immune cells at 48 h postinjections, selectively increasing monocyte numbers. Based on these results, SD-33 may have the potential to be used as an agent to decrease the negative effects of stress during weaning in pigs. However, further investigation is needed to better understand the timing of effect, and to rule out any immunosuppressive effects, which would be detrimental to the animal's well-being.

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CHAPTER ONE:
Literature Review

Introduction

Commercial swine producers must raise and sell a large number of healthy and fast growing pigs to be economically profitable. The pig's average daily gain, meat grade, and percent yield are all prerequisites for profitability. Many management practices relating to biosecurity and herd well-being are put into place in order for swine producers to reach such goals (Market Segment Specialization Program, 2001). However, the weaning process continues to be a major setback for producers.

Symptoms of stress associated with weaning are first typically seen in pigs upon arrival to the nursery, where pigs are faced with new social, environmental, health, and nutritional factors. Presentation of these changes all at once can be very taxing to the pig and often a physiological response ensues. Weanlings can quickly become fatigued, having a suppressed immune system and a loss of appetite that results in a decrease in body weight and lean muscle (Feuchter, 2004; USDA, 2008). Depending on how each individual pig copes, these weaning stress symptoms may lead to post-weaning mortality, ultimately decreasing the collective profitable gain. The USDA's National Animal Health Monitoring System (USDA, 2008) reported that due to infection, trauma, or starvation (among other unidentified problems), there is 2.9% mortality rate in the nursery phase. Along with mortality impacts to the industry, treating sick pigs due to illness or stress also increases costs, slows pig growth due to the recovery period, and prolongs the time before the pig goes to market. These stress-associated mortality rates add to a large sum annually. Under average market conditions, improving the survival by one piglet per litter would be worth approximately $400 million annually to the U.S. swine

industry (USDA-CSREES W173 report, 2006). This statistic does not include the costs of labor, medication, and lost weight for animals that are affected by weaning but survive.

This literature review will cover background information regarding the physiological effects the pig encounters during weaning as well as potential factors that can be used to assist the pig during this time. Understanding physiological pathways affected during weaning are necessary to design strategies to relieve negative effects of weaning-related stress. Specifically, this research focuses on strategies to increase feed intake and body weight and modulating immune responses during the post-weaning period by utilizing the tri-peptide opioid agonist Syndyphalin-33 (SD-33). The long-term goal of this work is to improve the well-being of the newly weaned pig.

Stress and Weaning

Management of weaning-related stressors includes monitoring environmental changes (such as temperature, humidity), management practices (such as castration, artificial rearing, early weaning), nutrition, social interactions (including aggression), and other factors that can adversely affect the health and well-being of newly-weaned pigs. The piglet's biological response to these environmental, management, nutritional, and social stressors is multifaceted and complex; and it affects the operation of many organ systems. Stress can suppress homeostasis and anabolism causing a deficit in energy that may lead to poor growth, and in extreme cases, death.

Weaning stress triggers the neuroendocrine pathway, and the endocrine axes, as well as autonomic responses, are stimulated simultaneously. Different types of stress (physical vs. psychological, acute vs. chronic) produce different physiological responses. In order to

understand how to help piglets overcome stress, it is important to be familiar with the stress pathway. The following review of stress physiology is summarized from the work of Sapolsky et al. (2000), Eiler (2004), Sherwood et al. (2005), Aron et al. (2007), and Gardner and Nessinson (2007).

Within one minute of experiencing the stressor, the autonomic nervous system sends a necessary distribution of energy throughout the body to only the tissues and organs that require such. Specifically, stress activates the sympathetic nervous system and suppresses the parasympathetic nervous system, as reviewed by Sapolsky et al. (2000).

The sympathetic nerves, located in the thoracic and lumbar region of the vertebral column, send and receive signals through neuronal channels. Afferent neurons carry sensations such as heat, cold, or pain toward the brain, and efferent neurons trigger functional changes peripherally by innervating effector tissue (ie, smooth muscle, cardiac muscle, and glands). Neurotransmitters such as norepeniphrine and acetylcholine are transmitted through neuronal synapses regulating the degree of response to the stress stimuli. Regulated by the mega-cellular neurons in the paraventricular nucleus of the hypothalamus, signals project to the chromaffin cells of the adrenal medulla where catecholamines, such as epinephrine, norepinephrine, and dopamine, are secreted directly into the bloodstream. The parasympathetic nervous system has a tonic inhibitory effect, decreasing functions that are not necessary to maintain fight or flight situations.

These events cause physiological changes that may be noticed by swine producers. Up-regulated respiration, heart rate, blood pressure, and renal function are effects of the sympathetic

response. Parasympathetic effect inhibition is often manifested as a decline or termination in appetite.

Along with the nervous system response, the stress-stimulated endocrine hypothalamic-pituitary-adrenal axis (HPA) is also initiated (as reviewed by Eiler, 2004, and Aron et al., 2007). Initially, the endocrine axis releases corticotropin-releasing hormone (CRH) from the parvocellular neurons in the paraventricular nucleus of the hypothalamus. Neurons that produce CRH (along with vasopressin and oxytocin) project to the median eminence, which lead to the anterior pituitary. Oxytocin and vasopressin normally stimulate the posterior pituitary, but their projections to the median eminence, together with CRH, are what regulate the release of adrenocorticotropic hormone (ACTH) from the corticotroph cells in the anterior pituitary. For ACTH to be released into the bloodstream, it must be cleaved from the prohormone pro-opiomelanocortin (POMC) by the prohormone convertase-1 enzyme in the corticotroph cells of the anterior pituitary. The ACTH release acts on its receptors in the zona fasciculata cortex region of the adrenal gland to release glucocorticoids. Cortisol is the primary glucocorticoid in the pig.

Once cortisol is synthesized and secreted from the adrenal cortex, it is transported within the circulation either bound to carrier proteins (corticosteroid-binding globulin [CBG] and albumin) or in the unbound/free state (Gardner and Nessinson, 2007). The half-life of cortisol within the circulation is determined to some extent by its association with these binding proteins in that they prevent cortisol from being excreted by the kidneys. In particular, CBG has a high affinity, but low capacity for binding cortisol compared with that of albumin. Cortisol bound to CBG is considered to be biologically inactive, whereas the albumin-bound and unbound cortisol

is biologically available to the cell. Therefore, the biological impact of cortisol concentration within the circulation will depend on both total cortisol levels as well as the concentration of CBG in the bloodstream.

Free cortisol and CBG-bound cortisol travel through the bloodstream and bind to two types of receptors: mineralocorticoid and glucocorticoid receptors (Sherwood et al., 2005). Both receptors are steroid hormone receptors found in the cytoplasm of the cell; however the stress response is primarily driven thorough the glucocorticoid receptors. As the free and CBG-bound cortisol hormones circulate through various target tissues, free steroid binds specifically to intercellular receptors in the cytoplasm of the cells, hence, reducing the free steroid concentration and shifting the equilibrium to the disassociation of CBG-bound cortisol in the bloodstream. This complex is then transported into the nucleus, and interacts with the DNA of the cell. Subsequently, the hormone-receptor ligand complex causes an increase in the transcription of specific genes coding for enzymes that in turn affect cellular metabolism, such as inducing catabolism of protein within muscles.

Through this pathway, ultimately, protein in the muscle and bone are broken down, gluconeogenesis in liver occurs, and fat reserves are consumed (Sherwood et al., 2005). These events all contribute to the production of energy to facilitate the pigs' body to respond to stress. Constant mobilization of energy (i.e. the breakdown of glycogen, production of glucose, and breakdown of fats) may at some point create a state of negative energy balance in the young pig when it occurs at the cost of energy storage. The lack of available energy further compromises the immune system, already suppressed by cortisol. This is of major concern when transporting newly weaned pigs to the nursery, as the pigs are mixed with others potentially carrying viral

and/or bacterial infections (Holden and Ensminger, 2005). Furthermore, the chronic stress response theoretically enhances appetite because expending energy at the cost of energy storage eventually stimulates intake for the assimilation of new energy. However, throughout the weaning process, stress can also hinder digestive development due to the catabolic effects on enzyme production leading to gastrointestinal problems, which further contributes to poor growth (Holden and Ensminger, 2005).

Weaning Stress and Immune Response

During the weaning process, the naive pig comes into contact with other pigs and new environmental factors. If proper management is not practiced, proliferation and introduction of pathogens may occur more readily to the incoming herd (Kahn and Line, 2005). Weaned pigs also must build new social hierarchies, leading to aggressive behavior and further prolonging the stress response. In 2000 and 2006, the USDA's National Animal Health Monitoring System conducted studies on swine health and management practices from a random sample of swine production sites with 100 or more pigs in 17 States, representing approximately 94% of pig inventory in the United States (USDA, 2008). This report noted the highest disease rate for both years in one or more nursery pigs over a 12-month period were among piglets infected with *E. coli* and *Streptococcus* bacteria.

When young piglets are presented with foreign pathogens, whether transmitted by fighting or through contamination (living in unsanitary conditions), their immune system is stimulated. The immune system responses described below are summarized from Lancaster (2001), Sherwood et al. (2005), and Donaldson (2007). .

The first response to an antigen is considered humoral and innate. The three primary white blood cells (WBC) involved are macrophages (long-lived mature monocyte that engulf foreign material), lymphocytes (B- and T-cells), and neutrophils (very active but short-lived phagocytes). The initial line of immune cell communication is by direct contact with other cells/foreign objects through receptor attachment and stimulation. This contact stimulates the inflammatory response, a biological response promoting the removal the injurious stimuli while also initiating the healing process in the tissue. The inflammatory process is an important part of a healthy response to infection; however, if amplified and sustained (especially during a febrile response), inflammation can compromise the young pig's survival.

Pathogens bind receptors, such as the toll-like receptors, on activated macrophages derived from monocytes (Lancaster, 2001; Sherwood et al., 2005; Donaldson, 2007) The pathogen is then identified as "foreign," tagged, and coated with opsin proteins, which increase the rate of phagocytosis. As phagocytosis occurs, mast cells found in connective tissue secrete hormonal mediators, such as histamine and chemotaxins, causing vasodilation and increased blood flow to the area. Inflammation continues as more phagocytes, other leukocytes (i.e. neutrophils, basophils, monocytes, and lymphocytes), and clotting factors migrate to the tissue. The secretion of chemotaxins also causes neutrophils and more monocytes stored in the bone marrow to migrate into the infected area via diapedisis. Leukocytes begin to proliferate once they reach the infected area and phagocytosis increases until the foreign pathogen is destroyed. Inflammation ceases once the stimulus has been removed.

The second type of immune cell communication is through the secretion of immunohormones called cytokines, which are responsible for initialing the febrile response

(Lancaster, 2001; Sherwood et al., 2005; Donaldson, 2007). The presence of a pathogen quickly induces lymphocytes to secrete pro-inflammatory cytokines, which reach the preoptic area of the hypothalamus and signal the release of prostaglandins (PGE_2), which in turn act locally to increase body temperature. Pro-inflammatory cytokines, important in producing fever, include interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNFα).

Tumor necrosis factor alpha acts on the hypothalamus by stimulating the HPA axis (described above), further stimulating the stress response. Cortisol stimulates the liver to secrete a large number of acute phase reactants such as C-reactive protein, serum amyloid-A, and fibrinogen in response to inflammation, as well as enhance the expression of IL-6 receptors in the hepatocytes of the liver. Cortisol also promotes IL-6 synthesis in the muscle and fatty tissue, which stimulates energy mobilization and leads to an overall increased body temperature. Interleukin-1 facilitates the migration of leukocytes to the sites of infection and also raises the set point in the hypothalamus thermoregulatory center to a higher temperature, resulting in fever. This elevated body temperature creates an unfavorable environment for bacterial growth while enhancing the innate immune response. While fever creates a preferred environment for immunological reactions, excessive fever can be detrimental to the well being of a young pig, leading to wasting and/or dehydration.

The lipopolysaccharide (LPS) model of immune challenge is often used when conducting studies to measure immune competence in livestock (Johnson and von Borell, 1994; Goujon et al., 1995; Mazzocchi et al., 1995). A large molecule composed of only the structural outer membrane component of Gram-negative bacteria, LPS acts as an endotoxin and initiates the inflammation process and subsequent immune reactions and behavioral effects. Specifically in

the pig, LPS has been documented to induce fever (Johnson and von Borell, 1994; Parrott and Vellucci, 1998) and reduce feed intake (Webel et al., 1997), typical symptoms and behavior of a pathogenic infection. At the cellular level, LPS has been shown to lower circulating levels of insulin-like growth factor –1 (IGF-1; Hever et al., 1997), and elevate IL-6, TNFα, and cortisol levels (Johnson and von Borell, 1994; Webel et al., 1997).

The LPS model is a satisfactory immune stimulant in most livestock. However, it does not completely demonstrate the physiological changes observed in pigs infected with enteric-type disease, frequently encountered during the weaning process. The duration of the effect of LPS on the immune system is fairly short (Parrott et al., 1995; Parrott and Vellucci, 1998). In contrast, enteric bacterial infections such as *Salmonella* and *E. coli* (both common diseases among piglets in 2000 and 2006; USDA, 2008) have a gradual onset and symptoms that can last for days (Wilcock and Schwartz, 1992).

Balaji and colleagues (2000) studied the effects of orally administered *Salmonella enterical* Typhimurim 3 x 10^9 cfu on the immune system in five week-old piglets. A gradual and sustained fever through five days post treatment was noted, unlike fever generated by LPS, which resolves within the first 12 hours post treatment (Johnson and von Borell, 1994; Webel et al, 1997; Balaji et al., 2000). Also, *Salmonella*-infected pigs displayed a prolonged surge of cortisol (Balaji et al., 2000), whereas a rapid recovery to baseline cortisol concentrations was observed in pigs injected with LPS (Webel et al., 1997). Surprisingly, PGE_2 and TNFα concentrations, which are increased during an LPS challenge (Parrott et al., 1995; Webel et al., 1997; Parrott et al., 1998), remained similar to that of the control groups suggesting that in

response to *Salmonella*, these hormones remained sequestered within the inflamed gut tissue and did not contribute to systemic increases leading to a fever (Balaji et al., 2000).

While similar results exist between an LPS and *Salmonella* challenge models, such as reduced feeding and growth (Balaji et al., 2000), an LPS model is lacking in demonstrating the complete endocrine profile of a weaned pig encountering an enteric and systemic infection.

Weaning Stress and Appetite

Sufficient feed intake is needed to enhance the performance, welfare, and health of the newly-weaned pig (Le Dividich et al., 2001). Pigs that handle stress poorly weigh less than their siblings at weaning and have a lower average daily gain. In extreme cases, stressed pigs can experience excessive energy loss and show signs of anorexia (Feuchter, 2004). Increasing feed efficiency will not only decrease the growth lag and market age (Kim et al., 2001; Feuchter, 2004), but will also aid in lowering housing, feeding, and other costs (Gempesaw and Halbrendt, 1991). Therefore, it is important to understand the physiology of appetite in order to efficiently improve feeding behavior. The following discussion of the appetite pathway is a summary from reviews by Forbes et al. (2001), Neary et al. (2004), Sherwood et al. (2005), and Wynne et al. (2005).

Many factors such as psychosocial, environmental signals, metabolic condition, and adipose concentration, may influence appetite. The major hormones involved in appetite regulation are leptin and insulin. Adipose tissue produces leptin, whereas insulin is a pancreatic hormone. Leptin and insulin circulate at levels proportional to body fat and exert effects in the hypothalamus. Both hormones stimulate and inhibit appetite centers of the hypothalamus, with the lateral nuclei controlling hunger, and the ventromedial area controlling satiety. Leptin is

reported to play a more central role than insulin for three reasons: 1) during food deprivation, plasma leptin concentrations decrease faster than body fat content, forming a more compensatory response before energy stores are substantially depleted, 2) leptin deficiency causes severe obesity, and 3) leptin acts as a long-term internal measure of energy state (Wynne et al., 2005).

In satiated or obese animals, higher levels of body fat and other energy reserves exist that increase the amount of leptin and insulin produced, leading to a reduction in food intake. Once produced, leptin crosses the blood brain barrier to stimulate the catabolic activity of POMC neurons and this increases expression of α-melanocyte-stimulating hormone (α-MSH) from the arcuate nucleus (Forbes et al., 2001). Alpha-MSH subsequently stimulates CRH from the paraventricular nucleus, an appetite suppressant and the major up-regulator of the HPA axis (discussed previously). Leptin further inhibits other potent appetite stimulants such as orexin, neuropeptide Y (NPY), and agouti-related peptide (AgRP; Sherwood et al., 2005). By decreasing orexin from the lateral hypothalamic area and from the perifornical area, the downstream effects of leptin (decrease in the secretion of NPY and AgRP in the arcuate nucleus) results in decreased feed intake, and if persistent, may lead to anorexia.

Alpha-MSH and AgRP are competing ligands for the MCR4 receptor. The AgRP antagonizes the action of melanocortin agonists, such as α-MSH. In rats, expression of AgRP mRNA in the arcuate nucleus of the hypothalamus is decreased subsequent to a stressful event (Kas et al., 2005). In turn, this increases the sensitivity of the receptor for α-MSH and a reduction in FI is observed (Kas et al., 2005). Emotional stress, such as restraint, and physical stress, as exemplified as forced swimming, in rats also increase the POMC gene expression and suppress feeding (Liu et al., 2007). When determining the degree of involvement by the

melanocortinergic pathway, antagonists to MC4R blocked the anorectic and anxiogenic effects of these stressors, suggesting that this pathway is heavily involved in stress related to anorexia (Liu et al., 2007).

Leptin also exerts effects on other physiological mechanisms. For example, leptin decreases the expression of endocannabinoids (Di Marzo et al., 2001), which are endogenous lipids that bind cannabinoid receptors. The amount of endocannabinoids produced in the hypothalamus is inversely correlated with the amount of leptin carried in the bloodstream. Marzo et al. (2001) found that the knockout leptin mice were not only obese, but also had higher than normal levels of hypothalamic endocannabinoids.

During feed restriction, lipid stores in adipocytes become reduced, decreasing the production of leptin and insulin (Sherwood et al., 2005). Therefore, appetite-stimulating peptides are not suppressed and feed intake is stimulated. This system would be beneficial to the weaned pig, given the significant decrease in feed intake following weaning (Feuchter, 2004). However, a pig undergoing weaning-related stress on average initially does not eat, suggesting that the physiological response to stress overrides any signal to increase intake (Feuchter, 2004; Sherwood et al., 2005).

An additional component of appetite is ghrelin. Ghrelin, secreted from the stomach and intestinal tract, is involved in promoting short-term feed intake in anticipation of an expected meal (Neary et al., 2004; Wynne et al., 2005). Its actions are exerted in the hypothalamus (at the arcuate nucleus) where it induces NPY and AgRP release (Wynne et al., 2005). In recently-weaned pigs, exogenous ghrelin administration stimulates the growth hormone (GH), insulin,

and cortisol axes (Wynne et al., 2005), decreasing weaning anorexia and increasing body weight gain.

Weaning Stress and the Growth Axis

Another aspect in controlling an efficient growth rate of piglets is through modulation of growth hormone (GH). Growth hormone is the primary hormone responsible for regulating overall body growth shifting energy stores from fat to muscle and bone. Specifically, GH stimulates protein synthesis and amino acid uptake by the muscle tissues to promote muscle growth, while promoting lipolysis and inhibiting lipogenesis in adipose tissue (Sherwood et al., 2005). Effects of GH include increased gluconeogenesis in the liver and decreased protein catabolism in muscle (Sherwood et al., 2005). The following discussion of this pathway is a summary of Nakazato et al. (2001), Sherwood et al. (2005), Aron et al. (2007), and Gardner et al. (2007).

Growth hormone is a 22-kDa protein released from somatotroph cells in the anterior pituitary in response to stimulation by cAMP. Growth hormone is stimulated by growth hormone releasing hormone (GHRH), which is secreted from the hypothalamus, specifically, the arcuate, paraventricular nucleus and supraoptic nucleus. The release of GHRH is stimulated by eating, exercise, or slow-wave sleep (Sherwood et al., 2005).

Regulation of GH occurs through a number of feedback mechanisms. Many mechanisms affect the secretion of GH, but this study focuses on appetite and muscle/bone stimulation processes. Ghrelin, stimulated by hunger, enhances GH secretion by 1) acting directly on the anterior pituitary and 2) acting indirectly on the hypothalamus to increase GHRH (Nakazato et

al., 2001). Together with GHRH, adenyl cyclase is stimulated and GH production from the anterior pituitary is up-regulated (Nakazato et al., 2001).

Growth hormone is inhibited by growth hormone inhibiting hormone. Growth hormone inhibiting hormone is secreted from the arcuate, paraventricular nucleus, and supraoptic nucleus of the hypothalamus, and exerts its affects on the neurotransmission and cell proliferation of GH via interaction with G-protein-coupled somatostatin receptors (Aron et al., 2007). Growth hormone also inhibits its own release, in order to maintain an efficient balance, by decreasing GHRH levels secreted from the hypothalamus (Sherwood et al., 2005).

Peripheral receptors for GH are cytokine receptors, that when activated, dimerize and become phosphorylated forming homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators to produce insulin-like growth factor, IGF-1 (Gardner and Nessinson, 2007), which binds receptors to regulate somatic growth and cellular proliferation (Sherwood et al., 2005; Gardner and Nessinson, 2007). Studies have shown that IGF-1 may also serve primarily as the negative feedback signal to the hypothalamus and anterior pituitary to decrease GH production (Roith et al., 2001).

Growth hormone also plays an important role in immunity, as GH receptors are found on certain WBC (lymphocytes, monocytes and neutrophils) as well as lymphatic tissues. Growth hormone stimulation of lymphocytes enhances antibody synthesis and basal lymphocyte proliferation (as reviewed by Kelley, 1990). Growth hormone also acts on phagocytic cells by activating the superoxide release mechanism of macrophages (derived from monocytes), which is an oxygen-dependent killing mechanism against invading pathogens (as reviewed by Kelley, 1990). Granulocyte differentiation of neutrophils occurs more readily upon both GH and IGF-1

stimulation (as reviewed by Kelley, 1990). During the stress response, as discussed previously, raises in cortisol postpones anabolism, disrupting growth in young animals. Typical of the endocrine profile observed during undernutrition (Vance et al., 1992; Straus, 1994), weaning increases concentrations of growth hormone (GH) and decreases serum concentrations of IGF-1 and IGF-2 (Carroll et al., 1998) in piglets.

Opioids and Stress

Cortisol levels increase at weaning, as discussed previously, due to stressors such as new environmental, social, housing, and food changes (Kojima et al., 2008; Cooper et al., 2009). Therefore, the neuro-endocrine HPA axis is also modified during weaning, which stimulates the up-regulation of POMC, the precursor for ACTH and endorphins.

Opioid involvement in the stress pathway appears to be species- and receptor-dependent. Pascoe et al. (2008) observed elevated cortisol concentrations in monkeys, and identified the κ-opioid receptor as the most responsive to stimulation and upregulation of both cortisol and ACTH. In humans (Pechnick, 1993), as well as in monkeys (Pascoe et al., 2008), μ-opioid receptor agonists inhibit or have no effect on the HPA axis. However, in rodents, the oposite is true (Nikolarakis et al., 1987). In post-pubertal gilts, treatment with a μ-opiate receptor antagonist (naloxone) increased cortisol levels (Barb et al., 1986).

Opiates and Immune Function

The stress of weaning can lead to an immunosuppressed state, leaving the piglets more susceptible to disease, as discussed previously. Opioids can modulate immune function directly or indirectly, through actions in peripheral tissue such as the adrenal gland or pituitary, or in the nervous system (Sibinga and Goldstein, 1988). Opioids can modulate immunological response to

tissue injury, microbial invasion, and inflammation. The traditional view that opioids are primarily immunosuppressant in nature is derived from experiments using supraphysiologic or chronic doses. Opioids actually produce a variety of effects depending on receptor recognition, strength, and duration of dose.

Evolutionarily, opioids originated as immune signaling molecules for microbial invasions (Stefano and Kream, 2008). Both invertebrate and vertebrate proenkephalins contain an antibacterial peptide called enkelytin (Goumon et al., 1996; Stefano and Kream, 2008). This antibacterial peptide is active upon co-secretion with other immune signaling molecules from that opioid (Stefano et al., 1998) to instantly attack the pathogen while allowing time for immune recruitment.

Enkephalins are one of three major families of endogenous opioid peptides, which also include endorphins and dynorphins. Enkephalins are coded from the proenkephalin gene (PENK), and/or cleaved from the POMC product, which is also the precursor for ACTH and α-MSH. Therefore, in situations of stress where the level of ACTH is increased, levels of enkephalins also increase slightly. Once enkephalins are produced, they act as neurotransmitters and bind one or more of three main subtypes of opioid receptor (μ-, κ-, and δ-opioid receptors). Opioid receptors are not only located pre-synaptically, but also post-synaptic as well. Therefore, it is important to note that the actions of opioids can vary depending on the location within the body (Kandel and Schwartz, 1985). For example, a particular opioid may act as an antagonist to the κ-opioid receptors in the brain, and as an agonist at the same type of receptor in the large intestines.

Wybran et al. (1979) reported one of the first studies measuring opioid activity in the immune system of humans. This study showed that met-enkephalins have a stimulatory effect on the rosetting of blood lymphocytes, whereas morphine inhibits rosetting. This increase, in turn, increased the number of antigens recognized by the lymphocytes (Wybran et al., 1979). In a later study, mouse pre-proenkephalin mRNA and enkephalin peptides were expressed and secreted upon activation of T-lymphocytes (Zurawski et al., 1986).

Opioid peptide production due to pain is beneficial to immune cells during inflammation (Stein et al., 1990). Dominika et al. (2009) found that in mice, about 30–40% of stimulated immune cells that collect at an injured nerve express three opioid peptides: β-endorphin, met-enkephalin, and dynorphin A. Each is activated respectively upon the binding to μ-, δ-, and κ-opioid receptors expressed in nociceptors (Dominika et al., 2009) and inhibit impulse propagation of pain. Therefore, upon injury, immune factors sequester and kill invasive substances whereas opiates secreted from leukocytes decrease the pain associated with this activity.

Cells of the immune system (neutrophils, monocytes, and lymphocytes) also express opioid receptors (Sibinga and Goldstein, 1988; Cabot et al., 2001; Richter et al., 2001; Finley et al., 2008). Recent evidence (Finley et al., 2008) suggests that activation of κ-opioid receptors on immune cells may induce an anti-inflammatory response while activation of μ-opioid receptors induces a pro-inflammatory response.

Opiates have also been shown to influence factors involved in cytokine signaling mechanisms during inflammation. When presented with met-enkephalin at concentrations ranging from 1-100 nM, macrophages demonstrated enhanced superoxide production and

increased sodium and calcium uptake (Foris et al., 1986), all of which enhance transduction of cytokine signaling during inflammation.

In 1984, Van Epps and Saland found that human monocytes demonstrated positive chemotatic effects when presented with beta-endorphins and met-enkephalins. More than 10 years later, Grimm et al. (1998) also reported that both endogenous met-enkephalin and morphine induced human monocyte chemotaxis, but found that it inhibited chemokine-induced chemotaxis of human neutrophils. Increasing leukocyte chemotaxis upon an inflammatory stimulus will attract leukocytes to that specific site of inflammation more readily.

Opiates and Appetite Regulation

The growth lag seen among recently weaned pigs is well recognized and clearly associated with a reduction in feed intake (Bark et al., 1986; McCracken et al., 1995). Mu-opioid agonists stimulate appetite in many ways. Mu-opioids have been shown to up-regulate the activity of appetite stimulators such as AgRP (Hagan et al., 2001; Brugman et al., 2002) and NPY (Kotz et al., 1993; Pomonis et al., 1997; Dodo et al., 2005). They are also known to decrease the synthesis of certain appetite suppressors such as α-MSH, one product of the POMC gene (Wardlaw et al., 1996). Opioids can also decrease the expression of the MC4R.

Opiates and Growth

Opioids are known to stimulate GH secretion in rats when given centrally (Chihara et al., 1978) or peripherally (Krulich et al., 1986). Vaccarino and Taub (1997) provided evidence showing opioid feeding signals arise from the arcuate nucleus and are linked with the suprachiasmatic nucleus and medial pre-optic area of the hypothalamus. Downstream signaling initiates growth hormone releasing-factor (GRF) feeding mechanisms, thus, allowing for both

central and peripheral GRF integrations (i.e. appetite and growth). Further, research also indicates opiates stimulate GH signals which are transmitted through μ-opioid receptors and inhibited by κ-opioid receptors (Krulich et al., 1986), However, this pathway needs further investigation.

Opioid Antagonists

When measuring opioid-mediated activity, researchers have used the strong opioid antagonist naloxone (NAL; Sibinga and Goldstein, 1988). Naloxone binds opioid receptors with high affinity, out-competing opioids for access to the receptor active sites, and blocking receptor activation (Kosterlitz and Watt, 1968). Naloxone has its greatest affinity for the μ-opiate receptor; however, at high levels, it also has the ability to antagonize κ- and δ-opioid receptors (Sibinga and Goldstein, 1988; Taub et al., 1991; Kulkarni -Narla et al., 2001).

Syndyphalin-33

Syndyphalin-33 (SD-33) is a synthetic enkephalin designed and first synthesized and described by Kiso and coworkers in 1981. The technical annotation of SD-33 is Tyr-Dmet(O)-Gly-MPA, or more specifically, tyrosine-methionine sulfoxide-glycine-methylphenethylamide. Designed as a small functional fragment of met-enkephalin, SD-33 exerts prolonged analgesic activity (Kiso et al., 1981). It is important to note that specific actions of SD-33 are still largely unknown and may vary somewhat from the actions of enkephalins in general.

Recent SD-33 Research

Given the previous analyses, it is plausible that SD-33 may act as an appetite stimulator to increase feed intake, reduce the post-weaning growth lag, and help the piglet overcome the negative effects of stress.

Buonomo et al. (1991) reported that SD-33 stimulated GH secretion in rats, pigs, and sheep when administered by intravenous (i.v.) and subcutaneous (s.c.) injections and by oral gavage. Rats and hogs (40 kg) were treated with s.c. injections of SD-33. In rats, peak levels of GH were noted at 30 minutes post injection in a dose-dependent fashion. The authors observed an 8-fold increase in GH secretion within 30 minutes postinjection (~16 ng/mL) and a 2-fold increase by 90 minutes postinjection (~6 ng/mL) in hogs that received a single s.c. injection of 0.5 μmol/kg SD-33. Using an oral gavage of SD-33 in a rat model, GH levels peaked in a dose-responsive manner 30 minutes postinjection (1.5, 2.75, 5-fold by 1.0, 10, and 100 μmol/kg of SD-33, respectively) and remained elevated until 2 hours postinjection. In hogs given a 2.0 μmol/kg oral dose, GH concentrations were increased 2.2 fold within 30 minutes postinjection and remained elevated until 2 hours postinjection.

Overall, the response of GH to i.v. injections of SD-33 in sheep was immediate and transitory; however, the s.c. and oral administration resulted in a sustained increase in GH concentrations lasting approximately 1.5 hours for s.c. injections and 1-2 hours for oral administration. Both of these effects were blocked by naloxone (Buonomo et al., 1991).

Obese et al. (2007) used an adult wether model to determine the effect of SD-33 on feed intake. The authors observed that a single i.v. injection of SD-33 (0.05 and 0.1 μmol/kg) increased cumulative feed intake over a 48-hour period in a dose-dependent manner. The higher dose yielded increased feed intake over 24 hours relative to controls. No increase in feed intake was observed in wethers administered 0.1 nmol/kg SD-33 i.v. injected twice (five minutes apart), when concomitantly given 0.1 ug/kg LPS. The authors then determined that the ability of a

single i.v. injection of SD-33 to increase feed intake was attenuated by naloxone, and speculated that the actions of SD-33 may be mediated through the μ-opioid receptor.

These two experiments observe immediate of affect of SD-33 on GH concentrations but delayed effect (24-48 hours) on feed intake. Also, while this published data suggests that SD-33 is a μ-opioid based on the ability of naloxone to block its actions, the possibility that this molecule may also bind one of the other opioid receptor subtypes must be considered.

The experiments described hereafter attempt to characterize the effects of SD-33 on growth, appetite, and immune function in the newly-weaned pig.

CHAPTER TWO:
Effects of syndyphalin-33 on feed intake and circulating measures of growth hormone, cortisol, and immune cell populations in the recently-weaned pig

Abstract

The synthetic met-enkephalin syndyphalin-33 (SD-33) increases feed intake in sheep, and transiently increases circulating growth hormone (GH) concentrations in sheep, rats and pigs. Two experiments were performed to evaluate the effects of SD-33 on recently-weaned pigs. In a preliminary experiment, pigs were administered either SD-33 (0.5 μmole/kg, given IM) or saline immediately prior to a 3 h transport and subsequent placement into group pens. Treatment with SD-33 increased ($P = 0.01$) daily feed intake; cumulatively, pen intake over 7 d postweaning tended ($P < 0.06$) to be greater than in control pens. In the second experiment, pigs were weaned and fitted with jugular catheters. The following day, pigs were treated with either SD-33 or saline as described above. Transient increases ($P < 0.05$) in circulating concentrations of GH (at 1 and 1.5 h postinjection) and cortisol (at 3.5 and 4 h postinjection) were observed in pigs treated with SD-33 relative to controls. No difference in feed intake was observed between treatments over 4 d postinjection. Increased ($P < 0.05$) numbers of circulating neutrophils, lymphocytes and monocytes were observed in both treatment groups over 4 d postinjection, and treatment with SD-33 tended ($P < 0.07$) to selectively increase monocyte numbers. Although SD-33 has potential to be used to increase feed intake and decrease the negative effects of stress during weaning in pigs, further investigation is needed to better understand the timing of effect and to rule out possible immunosuppressive effects.

Introduction

Syndyphalin-33 (Tyr-D-Met(O)-Gly-N-methylphenethylamide; SD-33) is a synthetic opioid capable of prolonged analgesic activity (Kiso et al., 1981). In 1991, Buonomo and co-workers administered SD-33 (orally, subcutaneously and intravenously) to pigs, rats and sheep. They found that the SD-33 treatment resulted in transient increases of circulating concentrations of growth hormone (Buonomo et al., 1991). More recently, in 2007, SD-33 was found to increase feed intake (FI) in adult sheep 48 h after intravenous administration (Obese et al., 2007). This effect was lost when the animals were inoculated with a lipopolysacharide challenge at the time of SD-33 administration (Obese et al., 2007). In each of these three studies, the effects of SD-33 relating to analgesia, circulating GH concentrations, and FI were all blocked by naloxone, suggesting that the observed effects were mediated (at least in part) through the μ-opioid receptor. The discrepancy in the timing of onset of the various effects of SD-33, (rapid stimulation of analgesia and growth hormone, but a prolonged interval before feed intake was increased) is not yet understood, but may be due to difference in complexity of affected pathways and/or differences in age/species of the animal models used.

In recently weaned pigs, weaning stress leads to decreased FI, increased susceptibility to disease, and poor growth, all of which are well-documented (Matteri et al., 2000; Kojima et al., 2007; Kojima et al., 2008). These responses to the stress of weaning often result in a growth lag, ultimately delaying production gain and profitability. Stress can also contribute to severe illnesses and even mortality, particularly in conditions of sub-optimal herd health or management. Through its various effects, particularly on FI, SD-33 may offer some protection

during the weaning process by increasing overall health and well-being during this critical period.

Two experiments that investigate the potential for SD-33 to improve the post-weaning growth lag in recently weaned pigs. The first experiment describes a preliminary trial characterizing on the effects of SD-33 on FI and growth. The second experiment concentrates on the acute affects of SD-33 on the growth, stress, and immune axes.

Materials and Methods

Animals and Diets

All animal procedures were reviewed and approved by the University of Tennessee Animal Care and Use Committee. Crossbred pigs (Landrace x Duroc x Hampshire) were farrowed in standard farrowing pens and processed according to standard University of Tennessee Experiment Station practices at 4 to 7 d of age. Procedures for processing included needle teeth clipping, tail docking, iron supplementation, ear tagging, and castration of males. The pigs were kept in farrowing pens with their dams until weaning, with creep feed (Diet 554PE, Tennessee Farmers Cooperative, LaVergne, TN) available 10 d after birth until weaning.

Experimental Design

Exp. 1. On d 0, 12 pigs (6 barrows and 6 gilts, 24 ± 1 d of age, 7.08 ± 0.22 kg) were weighed and randomly allocated by gender and BW to the following treatment groups: SD-33, receiving 0.5 μmole/kg SD-33 (Bachem, Torrance, CA) in saline by a single i.m. injection of 0.5 mL or less; or VEH, receiving a single i.m. injection of 0.5 mL saline. Pigs were removed from their sows, administered their respective treatments, mixed, and subjected to a 3 h transport in a vented, bedded livestock trailer. Upon return, 3 pigs were grouped by gender into pens (1.3 m^2),

2 pens per treatment group. Nursery feed (described above) and water were provided ad libitum. Pen FI and individual BW were recorded daily through d 7.

Exp. 2. Sixteen pigs (8 barrows and 8 gilts, 24 ± 1 d of age, 7.66 ± 0.08 kg) were removed from their dams, weighed and fitted nonsurgically with an indwelling jugular angiocatheter as described previously (Carroll et al., 1999). Briefly, an angiocatheter was inserted into the jugular vein while the pigs were immobilized with isoflurane for approximately 10 min. Immediately after catheter placement, pigs were placed in individual pens (1.3 m^2) with nursery feed and water provided ad libitum. Approximately 24 h following cannulation, pigs were weighed and allocated by gender and weight to 2 treatment groups (SD-33 and VEH) as described above. One gilt was removed from the study at this time due to failure of catheter patency. Serial blood sampling was performed such that 4 mL of blood was collected every 30 min for 6 h. Immediately following collection of the initial blood sample, pigs were administered their respective treatments. Blood (1 mL) was also collected at 0, 24, 48, 72 and 96 h postinjection for determination of circulating white blood cell (WBC) concentrations. The experiment was comprised of two replicates (8 pigs each).

Blood Collection and Analyses

For hormone assays, blood was collected into heparinized (90 USP sodium heparin) tubes and centrifuged at 2,000 x *g* for 10 min. The plasma was stored at –20°C until analyzed for GH and cortisol. For WBC count and differentials, blood was collected in tubes spray-coated with 5.4 mg of K_2 EDTA and immediately shipped on ice to a commercial clinical laboratory (Vet Path Labs, Tulsa, OK). The results reported are total WBC concentration (WBC/μL), which consists of the concentration of neutrophils, lymphocytes, and monocytes as well as the

percentage of neutrophils, lymphocytes, and monocytes relative to total WBC concentration. The total plasma cortisol concentration was determined by RIA as previously reported (Scroggs et al., 2002). Cortisol concentration was expressed as nmole/L. Intra- and inter-assay CV were 6.6 and 12.9 % for low (110 nmol/L), 8.3 and 4.8 % for medium (325 nmol/L), and 5.5 and 7.3% for high (772 nmol/L) cortisol standards. The sensitivity for the assay was 5.5 nmol/L. The plasma GH concentration (ng/mL) was determined by a commercially available RIA specific for porcine GH (Linco, St. Charles, MO). Intra- and inter-assay CVs were 17.6 and 19.8% for low (4.16 ng/mL), and 14.1 and 17.9 % for high (28.89 ng/mL) GH standards. The sensitivity of the assay was 1 ng/mL.

Statistical Analyses

The variables were analyzed with a mixed model ANOVA, using a model for a randomized block design. For Exp. 1, pen was the experimental unit for FI, and pig was the experimental unit for BW. In Exp. 2, pig was the experimental unit. For all variables except cumulative FI and gain, the model included treatment as a main effect with repeated measures. Square root transformations were performed on GH and cortisol data to preserve homogeneity of variance. For GH and cortisol, only animals with a complete set of data were included in the analysis (VEH: n = 7; SD-33: n = 4), as several cannulae failed during the sampling period. Least squares means were compared using Fisher's protected least significant difference. A significance level of $P < 0.05$ was used for all testing; trends where $P < 0.10$ were also reported. All graphical and textual descriptions of results are reported as raw means and standard errors. For Exp. 2, preliminary analysis detected no replicate effect, and so replicate was removed from the model.

Results

Feed Intake and Growth

Exp. 1. The treatment of the pigs with a single injection of SD-33 at the time of weaning resulted in increased ($P < 0.01$) daily FI (Figure 1A). Cumulatively, pen intake over 7 d postweaning in SD-33 pens (10.68 ± 0.58 kg) tended ($P = 0.06$) to be greater than in VEH pens (7.93 ± 0.41 kg; data not shown). Although weaning resulted in a loss of BW 1 d postweaning for both treatment groups ($P < 0.05$), BW did not differ between the 2 treatment groups at any time (Figure 1B).

Exp. 2. Feed intake of both VEH and SD-33 pigs increased over time ($P < 0.0001$), but did not differ between treatment groups at any time (Figure 2A). Cumulative FI was not different between SD-33 pigs (1.14 ± 0.16 kg) and VEH pigs (0.89 ± 0.11 kg; data not shown). There were no differences in BW due to treatment at any time (Figure 2B). Cumulative weight gain over 4 d postinjection did not differ between SD-33 pigs (0.81 ± 0.15 kg) and VEH pigs (0.96 ± 0.09 kg; data not shown).

Plasma GH and Cortisol

A transient increase ($P < 0.05$) in plasma concentrations of GH was observed at 1 and 1.5 h postinjection in the SD-33 pigs relative to the VEH group (Figure 3A). By 2 h postinjection, GH concentrations were not different between treatment groups and remained so for the remainder of the study. Plasma concentrations of cortisol were greater ($P < 0.05$) in SD-33 pigs at 3.5 and 4 h postinjection; by 4.5 h, cortisol concentrations in the two treatment groups no longer differed (Figure 3B).

Circulating WBC Populations

Overall, WBC concentrations, as well as individual populations of neutrophils, lymphocytes and monocytes, were elevated on d 1 to 4 postinjection relative to the pre-injection levels in both SD-33 and VEH pigs (Figure 4A-D). A trend ($P = 0.07$) was noted where monocyte numbers were increased in SD-33 pigs relative to the VEH pigs on d 1 to 4 postinjection (Figure 5D). This increase in monocytes resulted in a decrease ($P < 0.05$) in the percentage of WBC that were lymphocytes in SD-33 pigs (31.88 ± 4.76) relative to controls (47.87 ± 5.49) at 2 d postinjection (Figure 5B).

Discussion

The variables investigated in recently weaned pigs were feed intake (FI), body weight (BW), circulating levels of growth hormone (GH), plasma cortisol, and individual white blood cells (WBC). Each will be further discussed.

Feed Intake

The inhibition of piglet growth rate caused by weaning is well recognized and clearly associated with reduced FI (Bark et al., 1986; McCracken et al., 1995). In our preliminary experiment (Exp. 1.), a clear and robust increase in FI was seen in weaned and transported pigs that received a single injection of SD-33 at weaning. In agreement with observations made in adult sheep (Obese et al., 2007), this increase did not manifest until 2 d after administration. The continued increase in FI throughout the experiment is intriguing. While it is possible that this may arise out of a long-term stimulatory action of SD-33 on appetite, the mechanism behind the prolonged effect is unclear. In Exp. 2, no significant increase in FI was noted in treated animals, although a numerical pattern was evident. The pigs in the second experiment were administered

SD-33 the day after they were weaned and cannulated, and these pigs were not subjected to transportation. The amount, duration and type of stress experienced by the pig may all modulate the physiological response to SD-33.

The effects of SD-33 in FI and GH can be blocked by naloxone, leading to the speculation that SD-33 is a μ-opioid receptor agonist (Buonomo et al., 1991; Obese et al. 2007). Mu-opiates upregulate the activity of appetite stimulators such as agouti-related peptide (AGRP; Hagan et al., 2001; Brugman et al., 2002) and neuropeptide Y (NPY; Kotz et al., 1993; Pomonis et al., 1997; Dodo et al., 2005). They are also known to decrease the synthesis of certain appetite suppressors such as α-melanocyte stimulating hormone (α-MSH), one product of the proopiomelanocortin (POMC) gene (Wardlaw et al., 1996). Opioids can also decrease the expression of the melanocortin-4 receptor (MC4R), hence lowering its binding activity to AGRP and α-MSH (Chaki et al., 2005).

Stress, too, alters appetite regulators. In rats, the expression of AGRP mRNA in the arcuate nucleus of the hypothalamus is decreased subsequent to a stressful event (Kas et al., 2005). In turn, this increases the sensitivity of the receptor for α-MSH and a reduction in FI is observed (Kas et al., 2005). Emotional stress such as restraint and physical stress such as forced swimming in rats also increased the POMC gene expression and suppressed feeding (Liu et al., 2007). When determining the degree of involvement by the melanocortinergic pathway, antagonists to MC4R blocked the anorectic and anxiogenic effects of these stressors, suggesting that this pathway is heavily involved in stress related to anorexia (Liu et al., 2007). Taken together, these observations suggest that the effectiveness of SD-33 in increasing FI may depend on the severity of the stress the animal is experiencing at that time.

Appetite and Body Weight

For both Exp. 1 and Exp. 2, increases in BW did not accompany the increases in FI in SD-33 pigs. Thus, the elevated energy from the enhanced feed intake may be partitioned toward another physiological activity other than growth. It is important to note that opioids have systemic actions that include such processes as lipolysis in humans (Vettor et al., 1993) and in rabbits (Richter et al., 1983). Opioids are also known to increase metabolism in rats by an enhancing the release of triiodothyronine and thyroxine (Tal et al., 1984; Baumgartner et al., 1998). In the case of the newly-weaned pig, feed efficiency is not as much of an issue as is health and general well-being, particularly for the first few days after weaning.

Growth Hormone

Buonomo et al. (1991) observed immediate increases in GH levels in wethers (given 0.05, 0.10, or 0.20 μmole/kg SD-33 i.v.), rats (given 0.5, 1.0, or 2.0 μmole/kg SD-33 s.c.), and 50 kg barrows (given 0.50 μmole/kg SD-33 s.c.). This effect was also observed when 50 kg barrows were given SD-33 orally, but the increase was less intense and somewhat delayed compared to subcutaneous administration, where the peak GH concentration was observed as early as 30 min postinjection. In our study, treatment with SD-33 resulted in a transient increase in circulating concentrations of GH at 1 and 1.5 h postinjection. We observed increases of an amplitude similar to that reported for barrows treated with subcutaneous administration of SD-33, but the timing of the effect was more similar to the results reported for barrows administered SD-33 as an oral dose (Buonomo et al., 1991). We conclude that the route of administration may alter the profile of the acute response.

Vaccarino and Taub (1996) found evidence showing opioid feeding signals arise from the arcuate nucleus and are linked with the suprachiasmatic nucleus and medial preoptic area of the hypothalamus. Downstream signaling initiates growth hormone releasing-hormone (GHRH) feeding mechanisms, thus, allowing for both central and peripheral GHRH integrations (i.e. appetite and growth). Opioid-stimulated GH signals are thought to be transmitted through μ-opiate receptors and inhibited by κ-receptors (Krulich et al. 1986), further evidence that SD-33 works through μ-opiate receptors.

Cortisol

Cortisol is the primary glucocorticoid secreted during stress, involved in the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol also has widespread effects in other energy producing events such as promoting fat deposits (a desirable trait for swine yield), as well as in immune function, acting as an anti-inflammatory. Cortisol concentrations are normally increased for at least the first 24 h postweaning, presumably due to the stress associated with transportation, social mixing, and maternal separation (Kojima et al., 2008, Cooper et al., 2009). The baseline cortisol values in the current study (1 day postweaning) represent this elevated stress status.

We observed an increase in plasma cortisol concentrations in SD-33 pigs (relative to VEH pigs) at 3.5 and 4 h postinjection. Effects of exogenous opioids on the HPA axis appear to be species dependent. Pascoe et al. (2008) observed elevated cortisol concentrations in monkeys, and identified the κ-receptor as the most responsive to stimulation and upregulation of both cortisol and ACTH. In humans (Pechnick, 1993) and monkeys (Pascoe et al., 2008), μ-opioid receptor agonists inhibit or have no effect on the HPA axis. However, in rodents, the opposite is

true (Nikolarakis et al., 1987). In post-pubertal gilts, treatment with a μ-opiate receptor antagonist (naloxone) increased cortisol levels (Barb et al., 1986).

Some have speculated that μ-opioids exert effects on circulating cortisol concentrations through central regulation of corticotrophin-releasing hormone (CRH; Buckingham and Cooper, 1986; Nikolarakis et al., 1987). In our study, we did not observe an effect of SD-33 on circulating cortisol concentrations until 3 h postinjection. This may be a reflection of the time needed to translate an opioid's stimulatory effect on CRH in the hypothalamus (Yamauchi et al., 1997) through an increase in ACTH from the anterior pituitary before the end result, stimulation of cortisol release from the adrenal glands, can be observed.

Glucocorticoids also have a stimulatory effect on feed intake through their feedback to the hypothalamus. CRH-containing neurons release endocannibinoids, which act to increase feed intake upon stimulation of glucocorticoids (Dallman, 2003; Di et al, 2003). This may indicate yet another mechanism by which SD-33 acts to increase feed intake.

Immune Cell Populations

We observed increases in WBC, neutrophils, lymphocytes and monocytes over time in both SD-33 and VEH treatment groups. Previously, we have shown that circulating immune cell populations increase at 1 d postweaning and return to pre-weaning levels by 7 d postweaning (Kojima et al., 2008, Cooper et al., 2009), but we had not monitored these populations at any time in between. It would appear from our current data that immune cell populations continue to increase for several days postweaning. This may indicate prolonged inhibition of chemotaxis by cortisol and a progressive accumulation of cell numbers.

Treatment with SD-33 appeared to selectively increase monocyte numbers through 4 d postinjection, although the response did not reach statistical significance. Opioids are known to have immuno-modulary functions, and cells of the immune system (neutrophils, monocytes, and lymphocytes) express opioid receptors, as reviewed by Finley et al. (2008). Grimm et al. (1998) reported that endogenous met-enkephalin and morphine induced monocyte chemotaxis, but inhibited chemokine-induced chemotaxis of human neutrophils; these responses were blocked by naloxone. Recent evidence (Finley et al., 2008) suggests that activation of κ-opioid receptors may induce an anti-inflammatory response while activation of μ-opioid receptors induces a pro-inflammatory response. Naloxone, although primarily known as a μ-opioid receptor antagonist, also antagonizes κ- and δ-opioid receptors. While most of the published data suggests that SD-33 is a μ-opioid, the possibility that this molecule may also bind one of the other opioid receptor subtypes must be considered.

Implications

The synthetic enkephalin SD-33 has potential to be used as an agent to increase feed intake and decrease the negative effects of stress during weaning in pigs, but further investigation is needed to better understand the timing of effect, and to rule out any immunosuppressive effects which would be detrimental to the animal's well-being.

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APPENDIX

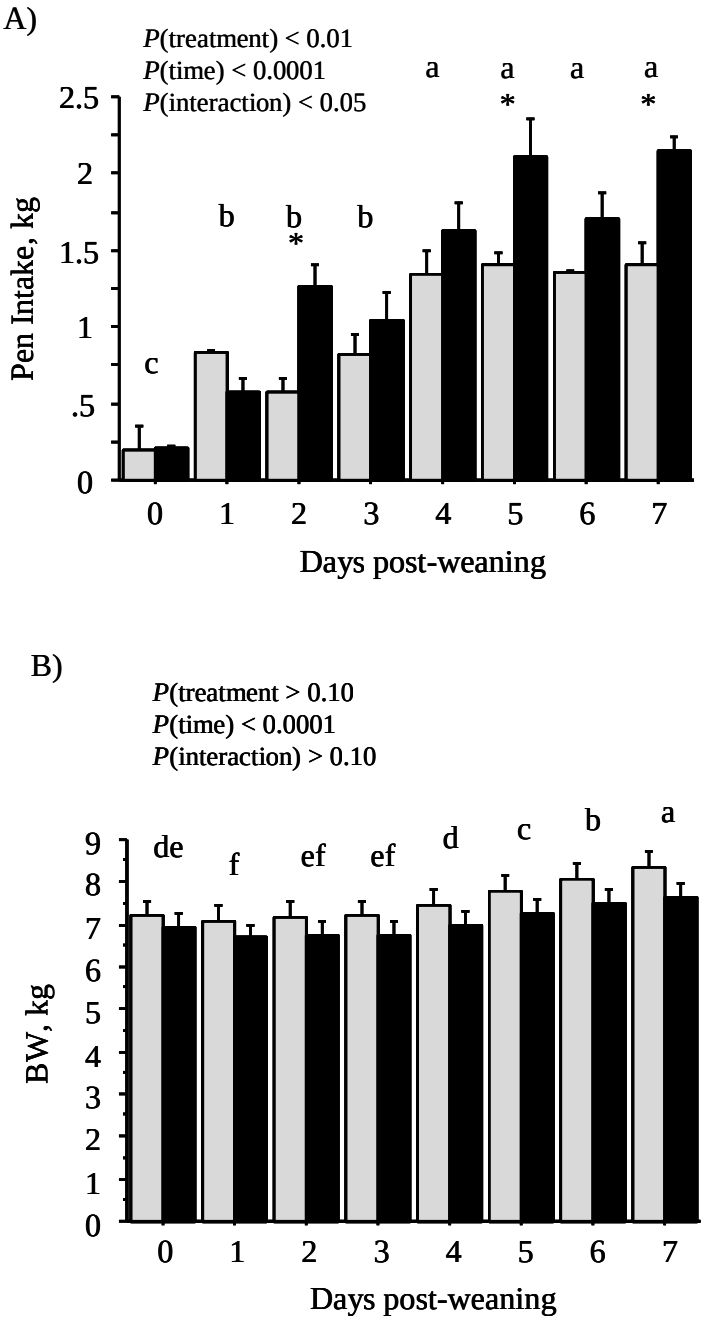
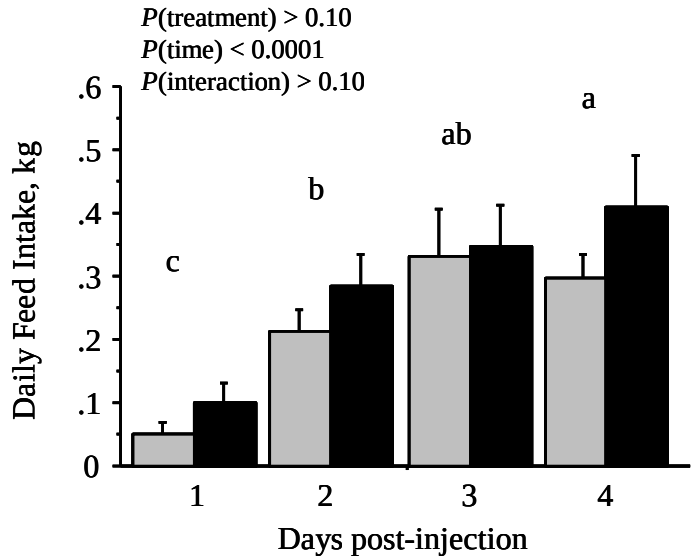


Figure 1. A) Feed intake in pens of pigs injected with saline (VEH; light bars) or 0.05 μmole/kg syndyphalin-33 (SD-33; dark bars) immediately prior to weaning (n = 2 pens). B) Body weight of VEH and SD-33 pigs (n = 6). For both graphs, raw means with SE are shown. $^{a\text{-}e}$Within a graph, means with different letters differ ($P < 0.05$). *Within a time point, means of VEH and SD-33 treatments differ ($P < 0.05$).

A)



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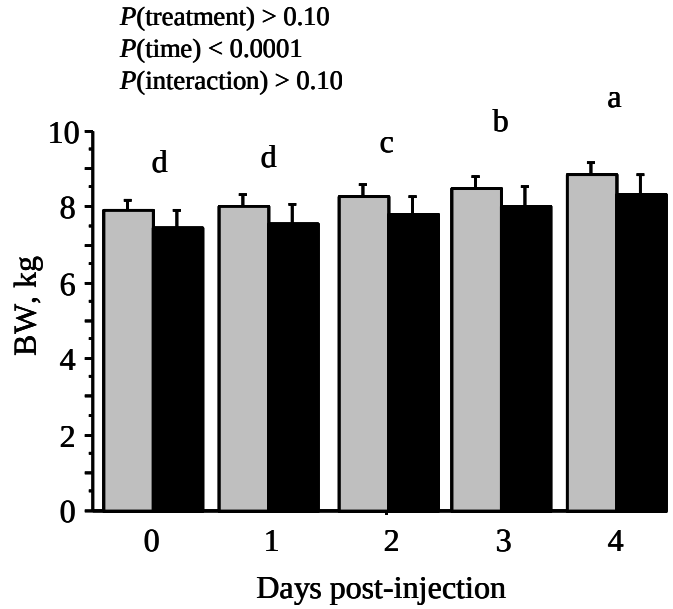

Figure 2. A) Feed intake of pigs injected with saline (VEH; n = 7; light bars) or 0.05 μmole/kg syndyphalin-33 (SD-33; n = 8; dark bars) 1 d after being weaned and fitted with jugular cannulae. B) Body weight of VEH (n = 7) and SD-33 (n = 8) pigs. For both graphs, raw means with SE are shown. [a-d] Within a graph, means with different letters differ ($P < 0.05$).

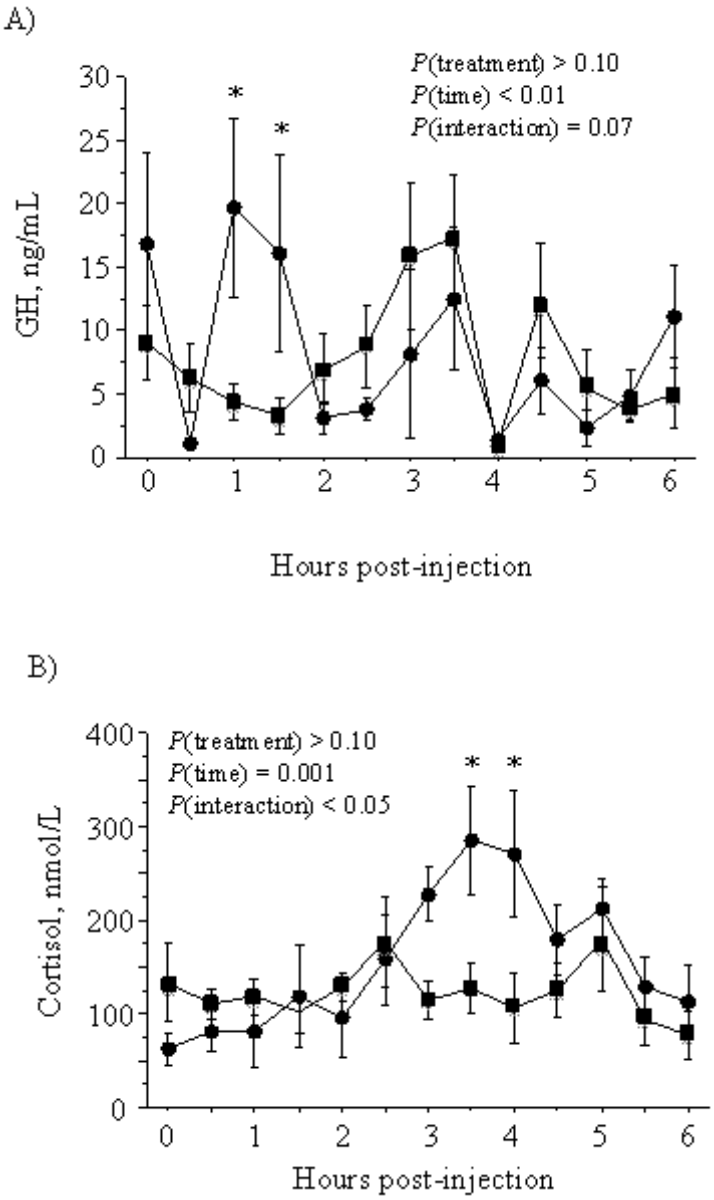

Figure 3. A) Circulating concentrations of A) GH and B) cortisol in plasma of pigs injected with saline (VEH; n = 7; ■) or 0.05 μmole/kg syndyphalin-33 (SD-33; n = 4; ●) 1 d after being weaned and fitted with jugular cannulae. Injections occurred immediately after time 0. For both graphs, raw means with SE are shown. *Within a time point, means of VEH and SD-33 treatments differ ($P < 0.05$).

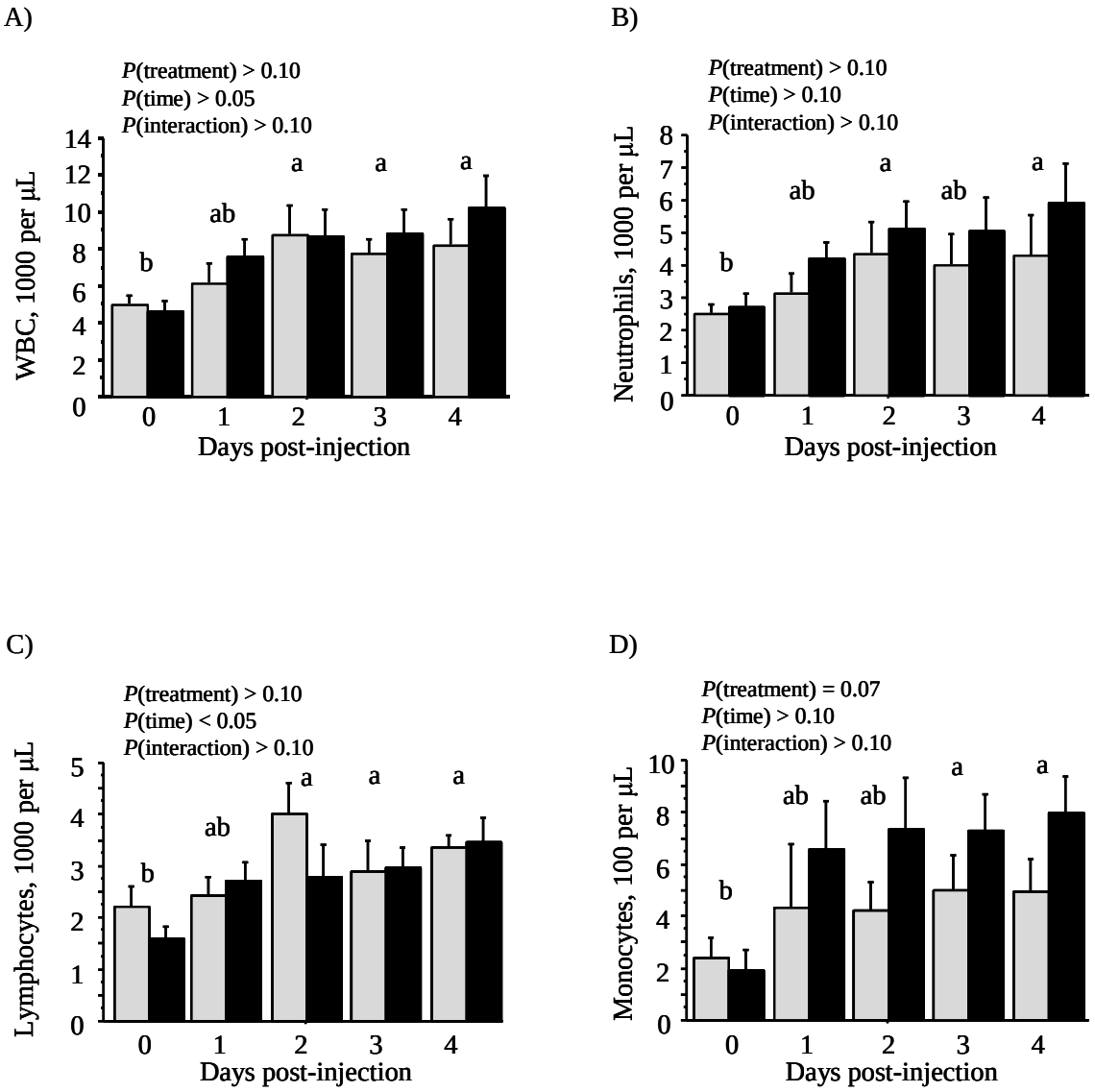

Figure 4. Circulating populations of A) total white blood cells (WBC), B) neutrophils, C) lymphocytes, and D) monocytes in pigs injected with saline (VEH; n = 7; light bars) or 0.05 µmole/kg syndyphalin-33 (SD-33; n = 8; dark bars) 1 d after being weaned and fitted with jugular cannulae. For all graphs, raw means with SE are shown. [a,b] Within a graph, means with different letters differ *(P* < 0.05).

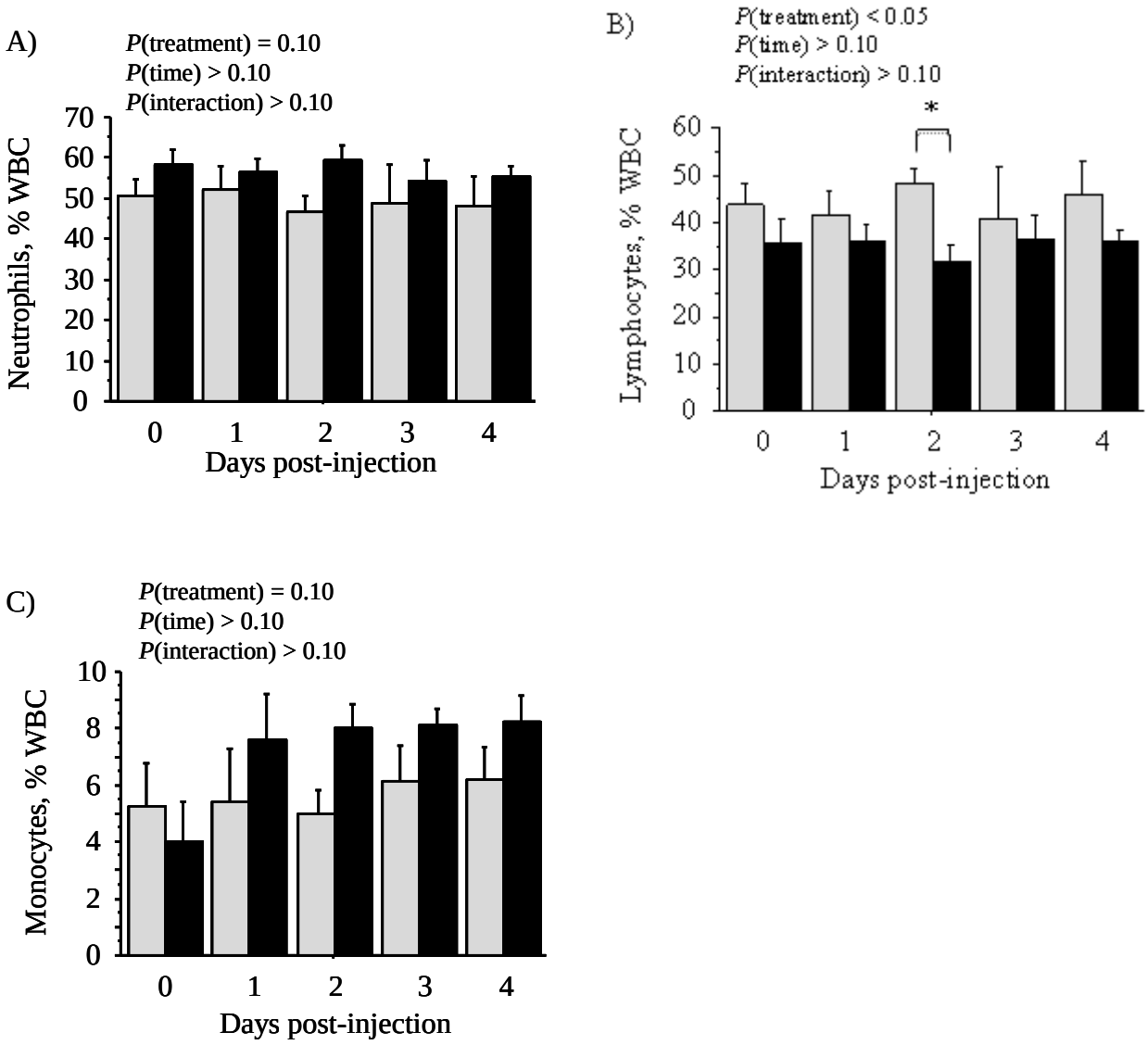

Figure 5. Percentages of total circulating white blood cells that are A) neutrophils, B) lymphocytes and C) monocytes in pigs injected with saline (VEH; n = 7; light bars) or 0.05 µmole/kg syndyphalin-33 (SD-33; n = 8; dark bars) 1 d after being weaned and fitted with jugular cannulae. For all graphs, raw means with SE are shown. * Within a time point, means of VEH and SD-33 treatments differ ($P < 0.05$).

CHAPTER THREE:

Effects of Syndyphalin-33 on immune function during a *Salmonella* challenge in recently weaned pigs

Abstract

This experiment was performed to evaluate the effectiveness Syndyphalin-33 (SD-33) has when recently weaned pigs are inoculated with the enteric pathogen, *Salmonella enterica* (SALM). On d 0, pigs (8 barrows and 6 gilts, 24 ± 1 d of age, 8.43 ± 0.82 kg) were weaned and fitted with jugular catheters. The following day, pigs were administered either SD-33 (0.5 μmole/kg, given i.m.) or SALM (oral dose of 5 x 10^9 CFU) as the treatment group, and either saline (VEH; 0.5 mL, given i.m.) or sterile broth (CON; 3 mL oral gavage) in a factorial arrangement such that 4 treatment groups existed: VEH+CON (n = 4), SD-33+CON (n = 3), VEH+SALM (n = 3), and SD-33+SALM (n = 4). There were no differences in feed intake (FI) or body weight (BW) among the SALM treated animals over time ($P > 0.05$). Cumulatively, FI among the SD-33+CON pigs was greater ($P < 0.05$) compared to the SD-33+SALM pigs. Over 4 d postinjection, white blood cell (WBC) populations increased ($P < 0.05$). On d 2 postchallenge, circulating neutrophils and lymphocytes were lower ($P < 0.05$) in VEH+SALM but not in SD-33+SALM pigs relative to VEH+CON and SD-33+CON pigs, demonstrating the ability of SD-33 to abrogate the affect of *Salmonella*; partial blocking was seen in both circulating neutrophil and lymphocyte populations. Also, on d 2 postchallenge, circulating monocyte populations were greater ($P < 0.05$) in pigs receiving SD-33 relative to controls regardless of *Salmonella* treatment. The results of this preliminary study suggest that the opioid SD-33 may act as an anti-inflammatory in recently weaned pigs.

Introduction

The process of weaning pigs involves many stressors, and the proliferation and introduction of a pathogen occurs more readily in new arrivals to the nursery facility (Kahn and Line, 2005). Syndyphalin-33 (Tyr-D-Met(O)-Gly-N-methylphenethylamide; SD-33) is a synthetic opioid with prolonged analgesic activity (Kiso et al., 1981), and has the ability to increase feed intake (FI) in adult wethers (Obese et al., 2007). The effect on FI was observed in wethers 48 h after intravenous administration of SD-33, but was not observed in wethers subjected to both SD-33 and a lipopolysaccharide challenge. Recently, we have observed increased FI in healthy recently-weaned pigs given a single intramuscular injection of SD-33 at weaning.

Exposure of pigs to the bacteria, *Salmonella enterica*, simulated a common immune challenge pigs frequently encounter during weaning (Baliji, et al., 2000). The typical lipopolysaccharide (LPS) challenge initiates a well-characterized inflammatory response and fever (Johnson and von Borells al. 1994; Parrott and Vellucci 1998), but the response is only quick and short-lived (Parrott et al. 1995; Parrott and Vellucci, 1998). The response to a *Salmonella* challenge, however, is gradual in onset, and sustained for up to 4 d (Wilcock and Schwartz, 1992). The USDA's National Animal Health Monitoring System stated in 2008 that such enteric diseases rank the highest among diseases frequently observed in weaned pigs (USDA 2008).

As an opioid agonist capable of stimulating appetite and inducing analgesia, SD-33 may offer some protection during the weaning process by increasing the overall health and well-being

of pigs during this critical period. Recent reports suggest that opioids may modulate immune function through altering chemotaxis and immune cell function.

The objective of this experiment was to characterize the effect of SD-33 on immune cell populations with and without a concurrent inoculation with a common enteric pathogen, *Salmonella*.

Materials and Methods

Animals and Diets

All animal procedures were reviewed and approved by the University of Tennessee Animal Care and Use Committee. Crossbred pigs (Landrace x Duroc x Hampshire) were farrowed in standard farrowing pens and processed according to usual University of Tennessee Experiment Station practice at 4 to 7 d of age. Procedures for processing included needle teeth clipping, tail docking, iron supplementation, ear tagging, and castration of males. The pigs were kept in farrowing pens with their dams until weaning, with creep feed (Diet 554PE, Tennessee Farmers Cooperative, LaVergne, TN) available 10 d after birth.

Experimental Design

Fourteen pigs (8 barrows and 6 gilts, 24 ± 1 d of age, 8.43 ± 0.82 kg) were removed from their sows, weighed and fitted nonsurgically with an indwelling jugular angiocatheter as described previously (Carroll et al., 1999). Briefly, an angiocatheter was inserted into the jugular vein while the pigs were immobilized with isoflurane for approximately 10 min. Immediately after catheter placement, pigs were placed in individual pens (1.3 m^2) with nursery feed and water provided *ad libitum*. Approximately 24 h following cannulation, pigs were weighed and allocated by gender and weight to 4 treatment groups in a 2 x 2 factorial design: SD-33+SALM

(n = 4), VEH+SALM (n = 3), SD-33+CON (n = 3), and VEH+CON (n = 4). Pigs in the SD-33 treatment groups received 0.5 μmole/kg SD-33 (Bachem, Torrance, CA) in saline by a single i.m. injection of 0.5 mL or less, and pigs in the VEH groups received a similar injection of 0.5 mL saline. Pigs in the SALM groups were inoculated with an oral dose of 5×10^9 CFU of live *Salmonella enterica* serovar Typhimurium culture in a volume of 3 mL (Strain 798-4232, National Animal Disease Control, USDA, Ames, IA) as described previously (Ebner et al., 2000 and Mathew et al., 2005). Pigs in CON groups received a 3 mL oral gavage of sterile broth.

Feed intake (FI) and body weight (BW) were measured and blood was collected daily at 0 (immediately prior to treatment), and 24, 48, 72 and 96 h postinjection.

Blood Collection Analysis

Blood for determining white blood cell (WBC) count and differentials was collected in tubes spray-coated with 5.4 mg of K_2 EDTA and immediately shipped on ice to a commercial clinical laboratory (Vet Path Labs, Tulsa, OK). The results reported are total WBC concentration (WBC/μL), which consists of the concentration of neutrophils, lymphocytes, and monocytes as well as the percentage of neutrophils, lymphocytes, and monocytes relative to total WBC concentration.

Statistical Analysis

The variables were analyzed with a mixed model ANOVA, using a model for a factorial design. Pig was the experimental unit. For all variables, the model included treatment (+/- SD-33) and challenge (+/- SALM) as main effects, along with the interaction between main effects, with repeated measures when appropriate. Initial body weight (weaning weight prior to cannulation) was included as a covariate for all analyses. Square root transformation was

performed as necessary to maintain homogeneity of variance. For variables presented as repeated measures, post-hoc analysis was conducted for each separate time-point (T-test). A significance level of $P < 0.05$ was used for all testing; trends where $P < 0.10$ were also reported. All graphical and textual descriptions of results are reported as raw means and standard errors.

Results

Feed Intake:

Daily FI increased ($P < 0.0001$) in each of the 4 d measured in all treatment groups (Figure 1A). Treatment with SALM resulted in an overall decrease ($P < 0.05$) in FI, particularly on d 3 post challenge ($P < 0.05$). An interaction occurred such that at 2 d post challenge, SD-33+SALM pigs consumed very little feed while SD-33+CON pigs consumed the greatest amount of feed ($P < 0.05$). Cumulative FI over the 4 d trial was decreased ($P < 0.05$) in SALM pigs relative to CON pigs (Figure 1B). There was a trend ($P = 0.065$) for a treatment interaction such that the effect of Salmonella was more evident in SD-33 (0.27 $\pm$ 0.09 kg) pigs than in VEH (0.66 $\pm$ 0.17 kg) pigs.

Body Weight

Cumulative weight gain was decreased ($P < 0.05$) in SALM pigs relative to CON pigs (Figure 2). A trend ($P = 0.067$) was observed such that SD-33+SALM pigs were less affected than VEH+SALM pigs, gaining 0.05 $\pm$ 0.27 and losing 0.13 $\pm$ 0.19 kg over the 4 d trial period, respectively.

Overall Immune Cells

Total WBC numbers increase ($P \leq 0.0001$) through d 4 (Figure 3). Populations of WBC were increased ($P \leq 0.0001$) in SD-33 pigs relative to VEH pigs. On d 2, WBC concentrations were lower ($P < 0.05$) in VEH+SALM pigs, but not SD-33+SALM pigs, relative to CON pigs.

Circulating neutrophil concentrations increased ($P < 0.0001$) over time and were higher ($P < 0.05$) in SD-33 pigs relative to VEH pigs (Figure 4A). Post-hoc analysis revealed that at 24 h postchallenge, VEH+SALM pigs had fewer ($P<0.05$) circulating neutrophils than did SD-33+SALM pigs. This pattern was also evident (but not statistically significant) at 48, 72, and 96 h. Although treatment did not affect the percentage of WBC that were neutrophils (Figure 4B), percentages tended ($P = 0.076$) to change over time, suggesting that neutrophil percentages across treatments were elevated at 24 h relative to the pre-challenge state.

Circulating lymphocyte concentrations increased ($P < 0.0001$) over time across treatment groups (Figure 5A). An interaction was observed such that at 48 h postchallenge, VEH+SALM pigs had lower ($P < 0.05$) numbers of circulating lymphocytes than did VEH+CON pigs. At 72 h, no effect of SALM was observed, but SD-33+CON pigs had greater numbers of circulating lymphocytes than did VEH+CON pigs. The percentage of WBC that were lymphocytes was not affected by treatment with SD-33 or SALM (Figure 5B). A trend was observed such that at 48 h postchallenge, SD-33+CON pigs had a lower ($P = 0.087$) percentage of WBC that were lymphocytes relative to VEH+CON pigs.

Circulating monocytes increased ($P < 0.0001$) over time across all treatment groups, and in contrast to lymphocytes, were elevated following SD-33 ($P < 0.001$) and SALM ($P < 0.05$) treatments, although the effect of SALM tended ($P = 0.082$) to be influenced by time (Figure

6A). It should be noted that circulating monocyte concentrations differed between treatment groups prior to treatment (0 h). Treatment with SD-33 tended ($P = 0.071$) to increase the percentage of WBC that were monocytes. The percentage of monocytes in SALM treated pigs increased ($P < 0.01$) as well (Figure 6B). The percentage of WBC that was monocytes decreased ($P < 0.005$) at h 24 relative to pre-treatment levels. The percentage of circulating monocytes then gradually increased at 72 and 96 h to become greater ($P = 0.005$) than 0 h levels..

Discussion

Feed Intake

As expected, FI was adversely affected by weaning in all treatment groups as seen 1 d post challenge (2 d postweaning). Pigs in all groups except SD-33+SALM recovered by d 2, and all groups were eating by d 3. At 2 d postchallenge, SD-33+CON pigs ate more than the other treatment groups, consistent with what we have observed previously in healthy pigs given SD-33 immediately prior to weaning (Kojima et al., 2009; Chapter 2). Inoculation with *Salmonella* markedly reduced FI, particularly at 3 d postchallenge. This reduction in FI is in agreement with previous studies which have shown decreased FI for up to 120 h postchallenge , depending somewhat on the dose given and the age of the pigs (Balaji et al., 2000; Jenkins et al., 2004). Simultaneous treatment with a single i.m. injection of 0.5 μmole/kg SD-33 did not increase FI in immune-challenged pigs. Similarly, Obese et al. (2007) also found that SD-33 did not counteract the reduction in FI due to an LPS challenge in adult wethers. The effects of SD-33 may not be potent enough to overcome the decreased appetite seen in an immune challenge; timing of injection or strength of dose may need to be altered.

Body Weight

Pigs challenged with *Salmonella* grew less over the 4 d trial period regardless of SD-33 treatment. Even though the interaction between SD-33 and SALM did not reach significance, VEH+SALM pigs lost weight relative to pre-trial BW, whereas the SD-33+SALM pigs did not. In the previous studies (Kojima et al., 2009; Chapter 2) no effect on BW was observed in healthy, weaned pigs treated with SD-33, in spite of increased FI. As opioids exert their effects on many physiological processes, including appetite and immune function, SD-33 may be altering the partitioning of calories toward some other function than growth.

Immune Cells

The primary function of WBC is to prevent and fight infections. In the young pig, its success in dealing with a pathogen may be dependent on the degree to which stress is affecting immune function. In the current study, the pigs were relatively stressed due to weaning and cannulation the day before. Neutrophil, lymphocyte, and monocyte numbers increased over time across all treatment groups. We have previously reported increases in total WBC count, neutrophils, lymphocytes and monocytes over time in newly-weaned pigs (Kojima et al., 2009; Chapter 2). Circulating immune cell population's increase at 1 d postweaning and return to pre-weaning levels by 7 d postweaning (Kojima et al., 2008, Cooper et al., 2009). It would appear that immune cell populations continue to increase for several days postweaning. This may indicate prolonged inhibition of chemotaxis by cortisol and a progressive accumulation of cell numbers.

Treatment with SD-33 increased circulating monocyte concentrations, most noticeably at 2 d postchallenge. We have previously shown that SD-33 tended to selectively increase the

number of circulating monocytes in healthy pigs given SD-33 immediately prior to weaning (Kojima et al., 2009; Chapter 2), although the response did not reach statistical significance.

Opioids are known to have immuno-modulary functions, and cells of the immune system (neutrophils, monocytes, and lymphocytes) express opioid receptors, as reviewed by Finley et al. (2008). Grimm et al. (1998) reported that endogenous met-enkephalin and morphine induced monocyte chemotaxis, but inhibited chemokine-induced chemotaxis of human neutrophils; these responses were blocked by naloxone. Recent evidence (Finley et al., 2008) suggests that activation of κ-opioid receptors may induce an anti-inflammatory response while activation of μ-opioid receptors induces a pro-inflammatory response. Naloxone, although primarily known as a μ-opioid receptor agonist, also is an antagonist to κ- and δ-opioid receptors. While most of the published data suggests that SD-33 is a μ-opioid, the possibility that this molecule may also bind one of the other opioid receptor subtypes must be considered.

An oral challenge of *Salmonella* would typically recruit circulating WBC to the gut, thereby decreasing circulating concentrations of neutrophils and lymphocytes. While this decrease in circulating cell numbers was observed at 48 h post challenge in VEH+SALM pigs, co-treatment with SD-33 appears to have blocked this effect. The exact mechanism for this is as yet undetermined, but may involve the inhibition of chemotaxis of neutrophils (and other WBC cell types) as described above.

In an intriguing examination of the effects of opioids, Stefano and Kream (2008) stated that opioids originated as immune signaling molecules for microbial invasions. Proenkephalins contain an antibacterial peptide called enkelytin (Goumon et al., 1996) that is co-secreted with

other immune signaling molecules to instantly attack pathogens while allowing time for immune recruitment (Stefano et al., 1998).

Implications

We have demonstrated that although co-treatment with SD-33 during an immune challenge did not result in increased feed intake, SD-33 exerted effects relating to circulating populations of immune cells. Further work is needed to determine if these effects are beneficial or harmful to the immune-challenged newly-weaned pig.

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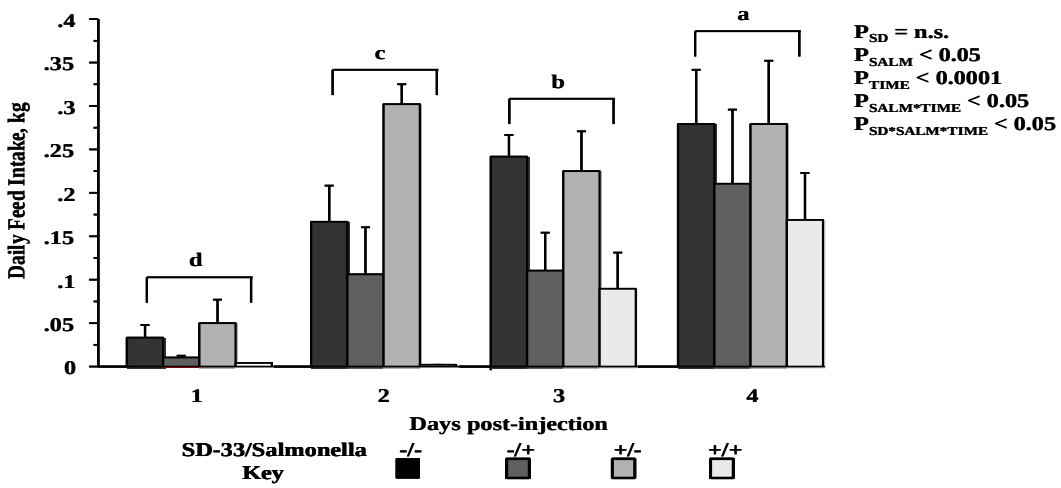
APPENDIX

A)



B)

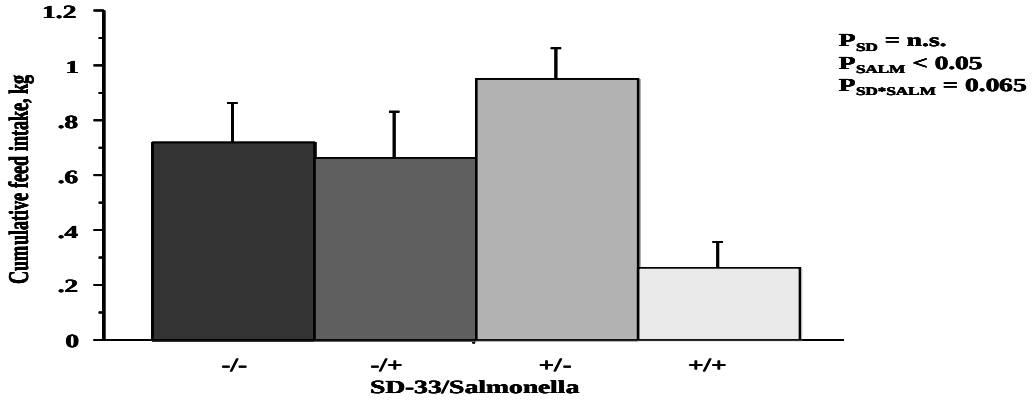


Figure 1: A) Daily and B) cumulative feed intake of pigs injected with SD-33 (0.05 μmole/kg syndyphalin-33) or saline, concurrent with an oral gavage of *Salmonella enterica* (5 x 10^9 CFU) or sterile broth. For both graphs, raw means and standard errors are shown. $^{a\text{-}d}$ Means of time points (across treatments) with unidentical letters differ ($P < 0.05$).

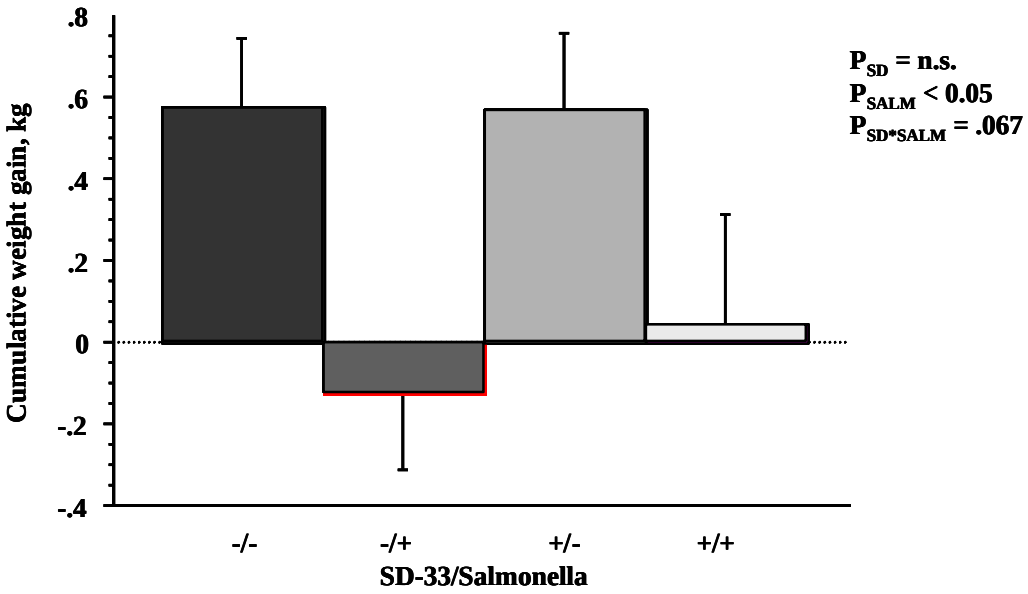


Figure 2: Cumulative weight gain of pigs injected with SD-33 (0.05 μmole/kg syndyphalin-33) or saline, concurrent with an oral gavage of *Salmonella enterica* (5 x 10^9 CFU) or sterile broth. Raw means and standard errors are shown.

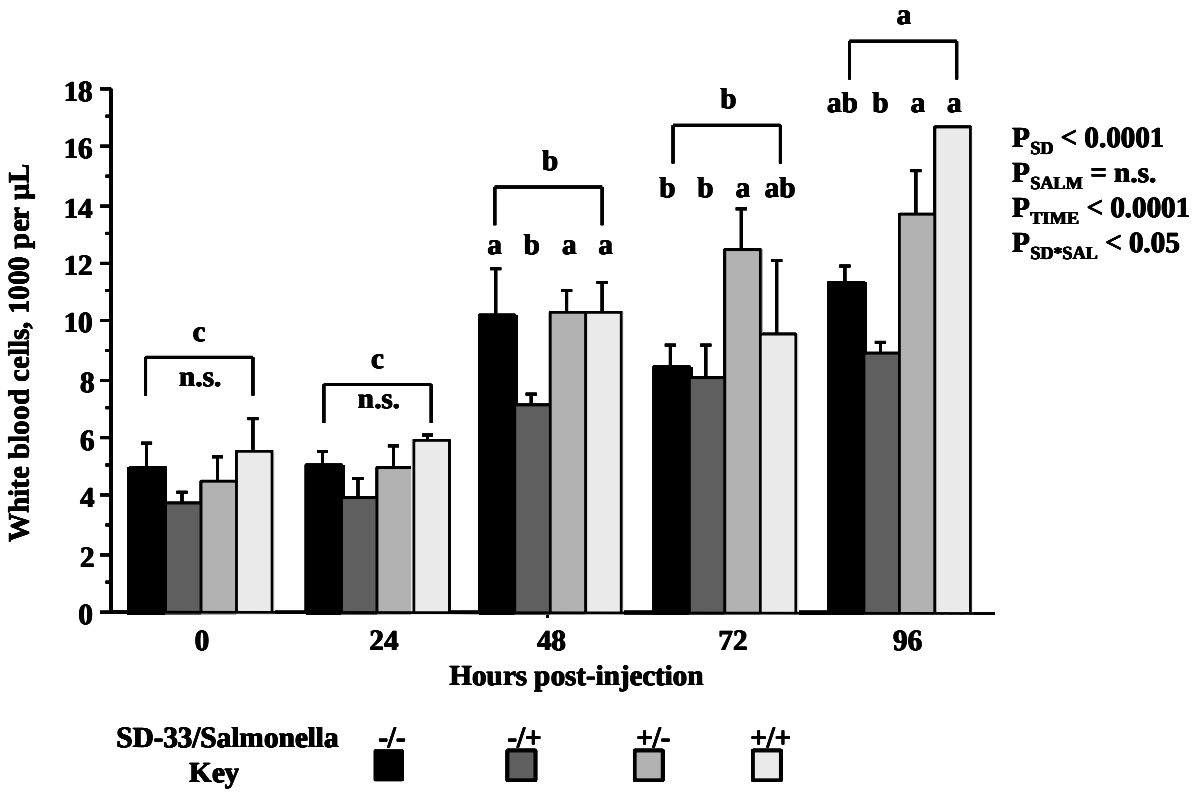
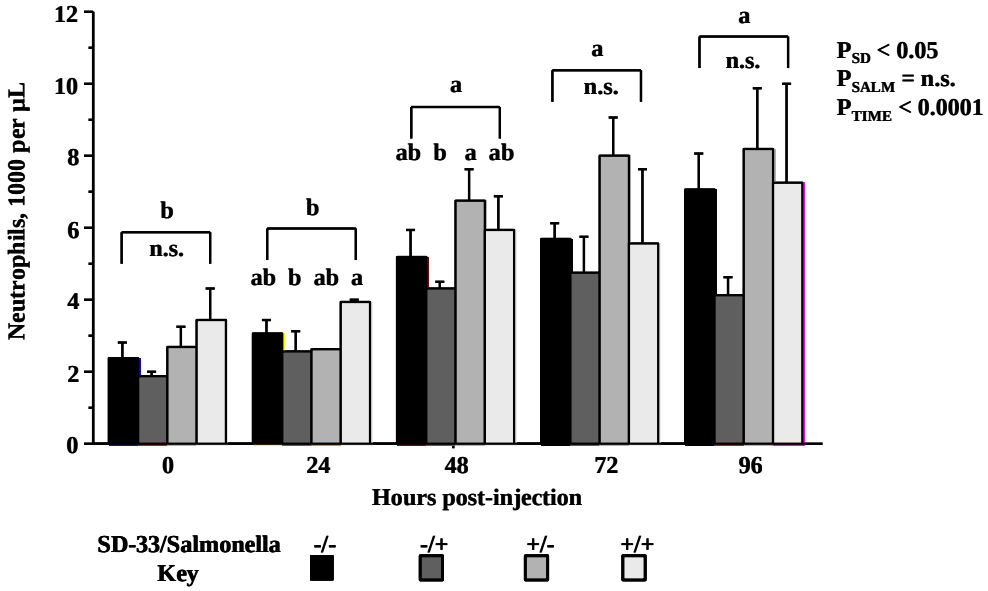


Figure 3: Total white blood cell count of pigs injected with SD-33 (0.05 µmole/kg syndyphalin-33) or saline, concurrent with an oral gavage of *Salmonella enterica* (5 x 10^9 CFU) or sterile broth. Raw means and standard errors are shown. [a-c] Above brackets, means of time points (across treatments) with unidentical letters differ *(P* < 0.05). [a-b] Below brackets, means of treatments (within a time) with unidentical letters differ (*P* < 0.05).

A)



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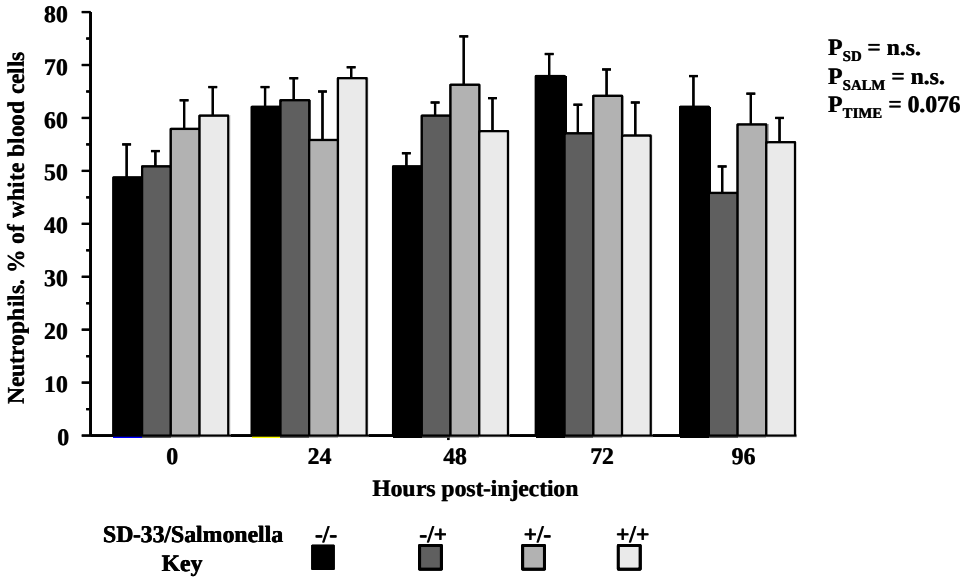
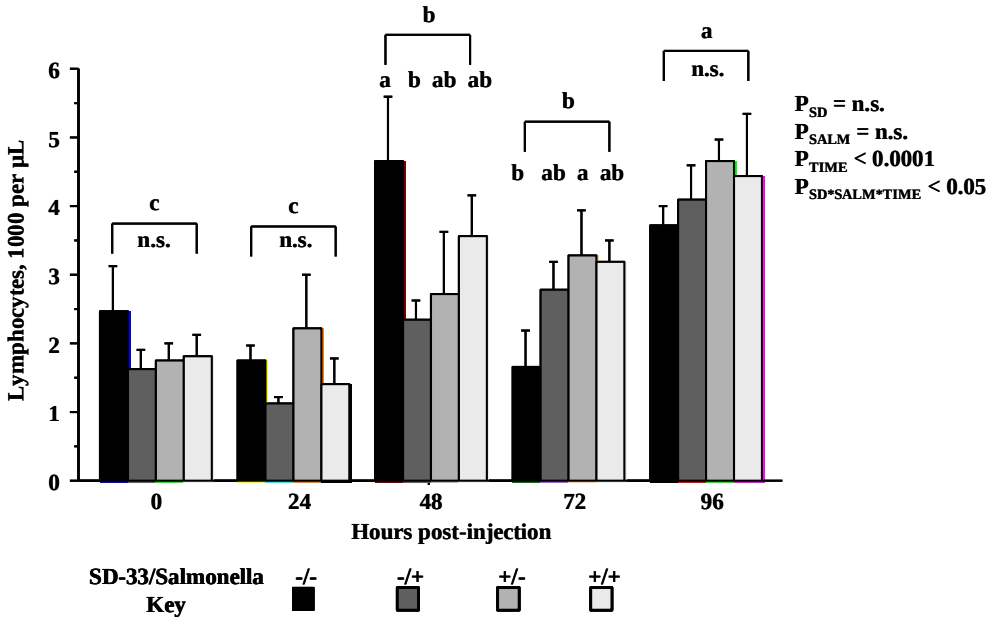


Figure 4: A) Absolute and B) percent neutrophils counts of pigs injected with SD-33 (0.05 µmole/kg syndyphalin-33) or saline, concurrent with an oral gavage of *Salmonella enterica* (5 x 10^9 CFU) or sterile broth. For both graphs, raw means and standard errors are shown. [a-b] Above brackets, means of time points (across treatments) with unidentical letters differ *(P* < 0.05). [a-b] Below brackets, means of treatments (within a time) with unidentical letters differ (*P* < 0.05).

A)



B)

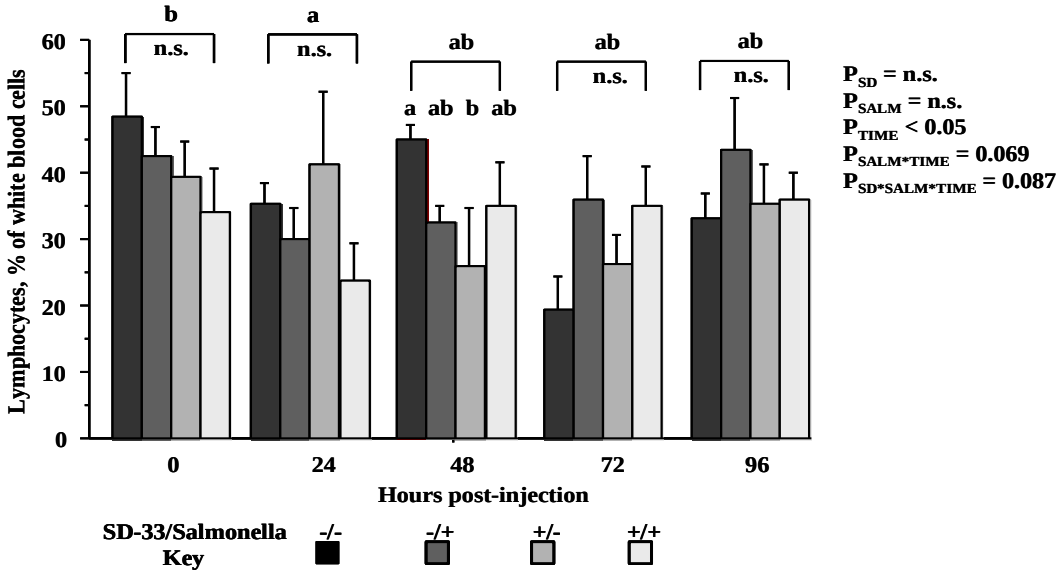
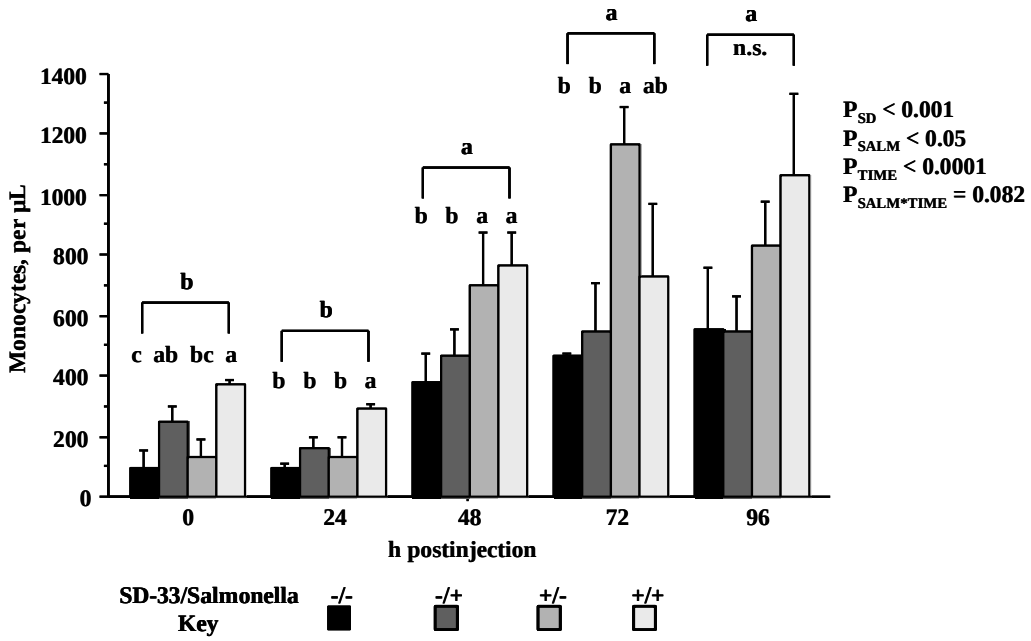


Figure 5: A) Absolute and B) percent lymphocytes of pigs injected with treatment allocations (SD-33 = 0.05 µmole/kg syndyphalin-33; SALM = 3 mL of $5x10^9$ CFU of live *Salmonella enterica*) over 4 days post-treatment. For both graphs, raw means and standard errors are shown. [a-c] Above brackets, means of time points (across treatments) with identical letters differ *(P* < 0.05). [a-b] Below brackets, means of treatments (within a time) with unidentical letters differ (*P* < 0.05).

A)



B)

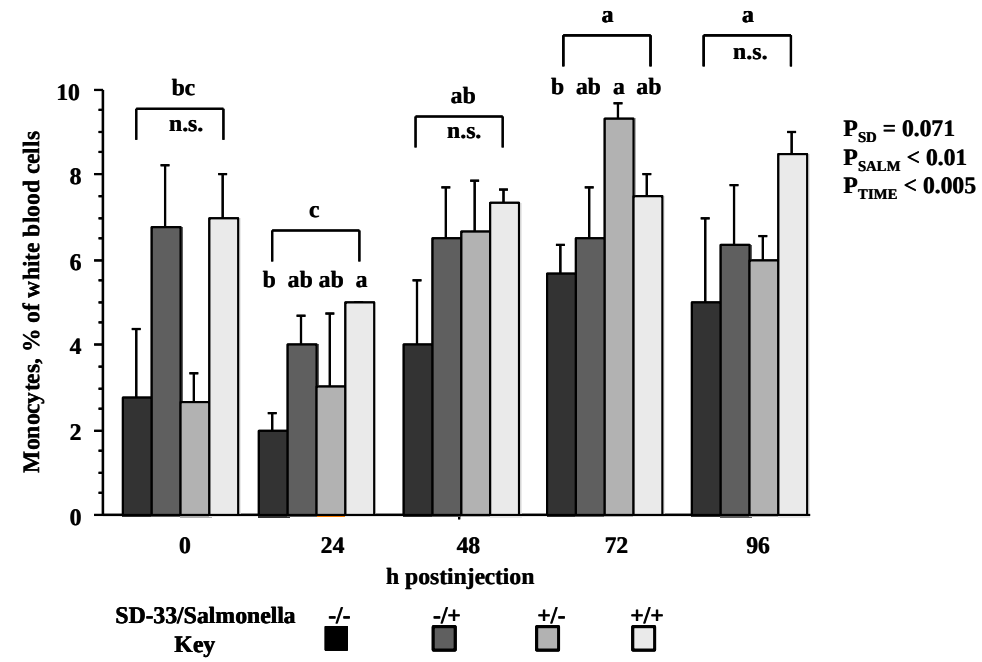


Figure 6: A) Absolute and B) percent monocyte counts of pigs injected with injected with treatment allocations (SD-33 = 0.05 µmole/kg syndyphalin-33; SALM = 3 mL of $5x10^9$ CFU of live *Salmonella enterica*). For both graphs, raw means and standard errors are shown. [a-c] Above brackets, means of time points (across treatments) with unidentical letters differ *(P* < 0.05). [a-b] Below brackets, means of treatments (within a time) with unidentical letters differ (*P* < 0.05).

CHAPTER FOUR
Conclusions and Implications

The results of this research has demonstrated that the synthetic enkephalin Syndyphalin-33 (SD-33) has potential to be used as an agent to increase feed intake and decrease the negative effects of stress during weaning in pigs. It has also demonstrated that, although co-treatment with SD-33 during an immune challenge did not result in increased feed intake, SD-33 exerted effects relating to circulating populations of immune cells, suggesting that this opioid may act as an anti-inflammatory in recently weaned pigs. Work is underway to confirm the anti-inflammatory effect. Further investigation is needed to better understand the timing of effect, and to rule out any immunosuppressive effects, which would be detrimental to the animal's well-being.

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Vita

Sarah Jo Jenkins was born in Nashville, TN on June 4, 1983 at 4:55pm. Her mother, Donna Jo Carey, and her father, Eugene Kenneth Jenkins, Jr, raised her in a Christian home. She is the youngest of three children with one older brother, James Grant Jenkins, and one oldest sister, Rachel Gene Parham. Currently, she has two nieces, Hannah Beth Parham and Julia Rachel Cain. She also has a step-father, Grant Lee Carey.

Throughout her teenage years, Sarah Jo played volleyball, softball, and track field events. She also participated in chorus, school plays, and played the violin and piano. She won awards for her artwork and creative writing pieces as well. In 2001, Sarah Jo graduated high school with honors at Pattonville High School in St. Louis, MO.

In 2007, Sarah Jo graduated Magna Cum Laude with a B.S. in Biology at Austin Peay State University. As an undergraduate, she received APSU's Jeanne Memorial Award and won 2nd place in the Tennessee Academy of Science for her original research in Animal Behavior, under the advisement of Dr. Andrew N. Barrass. Following her undergraduate degree, Sarah Jo was accepted as an intern in the Mammalian Genetics and Genomics division of Oak Ridge National Laboratory where she worked under Dr. Elissa Chesler.

In 2009, Sarah Jo graduated Summa Cum Laude with an M.S. in Animal Science and focus of Physiology at The University of Tennessee. She became a member of Gamma Sigma Delta, the honor society of Agriculture, that same year. Sarah Jo is currently pursuing a career in teaching.