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Effect of omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation to lactating sows on growth and indicators of stress in the post-weaned pig

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To the Graduate Council:

I am submitting herewith a thesis written by John M. McAfee entitled "Effect of omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation to lactating sows on growth and indicators of stress in the post-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.

Hank Kattesh, Major Professor

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DEDICATION

This thesis is dedicated to my parents, who have only ever encouraged me to do my best**.**

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ABSTRACT

The first experiment was designed to determine the concentration of protected fish oil product (**PFO**), as Gromega™, to be added that would sufficiently decrease the polyunsaturated fatty acid (**PUFA**) ratio in the sows' milk and colostrum. Of the 3 diets tested (0.25%, 0.5% and 1%) with a control (0% PFO), only the 1% PFO diet had an effect on the PUFA concentrations. The docosahexaenoic acid (**DHA**) concentration tended ($P = 0.05$) to be greater in the 1% PFO diet for both colostrum and milk samples. A second experiment was designed to examine the effects of feeding the 1 versus 0% PFO supplemented diet to sows on growth, markers of acute inflammation and stress in their offspring (16 piglets/treatment group) on d 0 (day of weaning) and d 1 and 3 postweaning. Piglets from sows supplemented the 1% PFO diet had greater gains in weight ($P = 0.03$) postweaning. These pigs also had a lower ($P < 0.01$) n-6:n-3 PUFA in the plasma when compared to piglets on the control diet. There was an overall treatment effect ($P = 0.02$) on plasma total cortisol, observed by lower concentrations in pigs on the 1% PFO diet. Plasma corticosteroid-binding globulin (**CBG**) concentrations were not different between treatment groups but were lower ($P < 0.001$) on d 1 and 3 when compared to d 0. The calculated free cortisol index [**FCI** (cortisol/CBG)] of pigs on the 1% diet was lower ($P = 0.02$) on d 1 and 3 when compared to the controls. The cytokines, IL-1β [beta], IL-6, and TNF-α [alpha] were measured following an ex vivo lipopolysaccharide (**LPS**) stimulation of monocytes and neutrophils in whole blood collected on d 0 and 1. Pigs on the 1% PFO diet tended to have a lower ($P = 0.098$) mean concentration of TNF-α in response to LPS when compared with that of the controls. These results suggest that providing a PFO supplement as 1% of the diet to sows

beginning in late gestation and during lactation can lower the n-6:n-3 PUFA ratio in their offspring, which may reduce the acute physiological stress response in the pigs postweaning.

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CHAPTER 1. INTRODUCTION

There are many challenges in a young pig's life that can lead to imbalance. Such challenges can cause strain and may arise in many forms. Beginning with entry into the world, a pig is subject to a barrage of all new experiences. The most trying time comes at weaning, in which a pig is housed with unfamiliar pigs of varying sizes and begin to form a hierarchy by fighting (Campbell et al., 2013). These stressors can incite a stress response, which is the body's reaction to a stimuli that offsets homeostasis that can lead to suppression of the immune system, weight loss and even death (Khansari et al., 1990; Salak-Johnson and McGlone, 2007). The stress response is characterized by stimulation of the hypothalamic-pituitary-adrenal (**HPA**) axis. Upon stimulations of converging neurons in hypothalamus, corticotropin-releasing hormone (**CRH**) is released, which in turn promotes production of adrenocorticotropic hormone (**ACTH**) in the anterior pituitary gland. The ACTH travels by way of the circulation to the adrenal gland and stimulates the release of glucocorticoids with the major glucocorticoid being cortisol in swine (Hicks et al., 1998). Circulating concentrations of corticosteroid-binding globulin (**CBG**), produced in the liver, control the bioavailability of circulating concentrations of plasma cortisol, with approximately 60-90% of the total cortisol in swine bound by CBG (Heo et al., 2005). The circulating CBG can be cleaved form cortisol by neutrophil elastase (Lee and Downey, 2001). The cleavage of CBG from cortisol, results in elastase-cleaved CBG which has a much lower affinity for cortisol than intact CBG, allowing freed cortisol to be biologically active (Nguyen et al., 2014).

Not long after weaning pigs begin fighting, the wounds inflicted produce an inflammatory response characterized by redness, pain, swelling, and loss of function (Myers et al., 2003). The white blood cells (**WBCs**) present at the site of inflammation release cytokines

tumor necrosis factor alpha (**TNF-α**) IL-6 and IL-1β, which induce vasodilation, increase blood flow, and increase the presence of adhesion molecules such as, vascular cell adhesion molecule-1 (**VCAM-1**), intercellular adhesion molecule-1 (**ICAM-1**), and E-selectins (Calder, 2006). The rise in adhesion molecules help to recruit increased numbers of the granulocytes and later monocytes and macrophages. The production of CBG by the liver can be affected by IL-6 and other cytokines during acute inflammation. Genes that produce CBG in hepatocytes are suppressed in the presence of IL-6, as seen in patients experiencing septic shock. (Emptoz-Bonneton et al., 2011). TNF-α and IL-6 have been shown to alter glucocorticoid sensitivity in cells of burn patients and those with major depression (Pace and Miller, 2009) Another consequence of increased concentrations of IL-6 and IL-1β is the increased production of acute phase proteins (**APP**) in the liver, such as C-reactive protein, fibrinogen, and haptoglobin (Emptoz-Bonneton et al., 2011). Haptoglobin has been proposed as a marker for inflammation and infection in the health status of swine (Chen et al., 2003)

Polyunsaturated fatty acids or PUFA are fatty acids with two or more double bonds in their carbon backbone (Sinclair et al., 2002). There are many different PUFA subtypes but this paper will focus on methylene-interrupted polyenes, specifically omega-3 (**n-3**) and omega-6 (**n-6**) fatty acids. These fatty acids contain two or more *cis* double bonds and are defined by the position of the first double bond relative to the methyl end of the molecule and comprise another broader category of PUFA named the essential fatty acids (**EFA**). As with essential amino acids, EFA are required by the body to function properly but must ingested by humans and other animals as they cannot be synthesized naturally (Leaf, 1996). Two particular EFA linoleic and alpha linolenic acid (**LA;18:2n-3 and ALA;18:3n-6**) are precursor molecules for desaturation

into eicosapentaenoic acid (**EPA;20:5n-3**) and docosahexaenoic acid (**DHA;22:6n-3**) for LA and arachidonic acid (**ARA;20:4n-6**) for ALA. The compounds EPA, DHA, and ARA are used by the body to produce eicosanoids and docosanoid species, as well as many other oxidative molecule species (Hong et al., 2003). They are also important in membrane fluidity and cell signaling (Koletzko and Rodriguez-Palmero, 1999).

One of the major mediators between inflammation and the stress response are a series of eicosanoids called prostaglandins, particularly prostaglandin E_2 (**PGE_2**). This prostaglandin is synthesized in the brain, near the hypothalamus, when IL-1β induces expression of cyclooxygenase-2 (**COX-2**) (Goshen and Yirmiya, 2009). The enzyme phospholipase A_2 cleaves ARA from the phospholipid membrane at the SN2 position (Calder, 2009). ARA is oxidized by a series of COX enzymes and prostaglandin synthases, mainly COX-2 and microsomal prostaglandin E synthase 1 (Furuyashiki and Narumiya, 2011). There are also a series of less potent prostaglandins, the 3 series, produced in the same manner. Instead of ARA being cleaved and oxidized, DHA or EPA are utilized (Calder, 2006). The EPA is oxidized to less inflammatory prostaglandin series 3 and DHA is oxidized to produce novel molecules like neuroprotectins and resolvins under the category docosaenoids, each act to lessen the effect that inflammation has on HPA activation (Calder, 2009).

Supplementation with n-3 PUFA and subsequent maternal transfer to the offspring has been shown in a variety of animals with beneficial effects (Hornstra, 2000; Yao et al., 2012). One of many sources for n-3 PUFA, protected fish oil (**PFO**) containing the n-3 PUFA EPA and DHA, can decrease the PUFA ratio (n-6:n-3) in the dams colostrum and milk. This leads to a subsequent decrease in the offspring's PUFA ratio (Fritsche et al., 1993; Gabler et al., 2007).

This reduction in PUFA ratio is accompanied by an increase in EPA and DHA concentrations which produce much less potent mediators of inflammation. The decrease in inflammation has been shown to reduce corticosterone concentrations in rats and mice (Kusnecov and Rossi-George, 2002; Ferraz et al., 2008; Yao et al., 2012).

The goal of the following study is to lower the n-6:n-3 PUFA ratio in pigs through supplementation of PFO to sows during late gestation and lactation and promote growth during postweaning. Through the reduction in this ratio, the expectation is that pigs will exhibit a reduction in total cortisol and the free cortisol index (**FCI**), which are indicators of stress, and the production of inflammatory markers, the APP haptoglobin and acute phase cytokines IL-6, Il-1β, and TNF-α.

CHAPTER 2. LITERATURE REVIEW

IMPACTS OF WEANING

Domestication and selective breeding in combination with swine management practices have reduced weaning ages of pigs to 3-4 wk, or earlier (Carroll et al., 1998). This allows swine operations to increase production and income by reducing the time pigs spend nursing and sows spend in the farrowing crates (Smith et al., 2006). However, weaning pigs at this age or sooner can be one of the most stressful times in the pig's life and can result in detrimental effects to its health, growth, and overall development (Hay et al., 2001). During the postweaning period, pigs experience multiple challenges that may consist of environmental, physical and social stressors. Stressors, such as separation from the sow, transportation and handling stress, social hierarchy stress, a change in diet, and increase in exposure to pathogens all contribute to an acute stress response in a pig upon weaning (Campbell et al., 2013). All of this may lead to poor performance and increased mortality if the pig cannot overcome these challenges (Colson et al., 2006). A study conducted by the Animal and Plant Health Inspection Service across 17 states that represented 94% of U.S. swine production in 2000 showed an average of 2.6% of nursery-age pigs died during a 6 month period. This means that on a farm weaning 2000 pigs over 6 months, 52 of those will die. Considering a live market price of $49.35/cwt and an average market weight was 278 lbs on Aug 8, 2016 meaning that if 52 pigs died that equates to $7,134.03 in loss and that is without accounting for pigs that take longer to achieve market weight due to postweaning challenges. Any pig that has reduced performance or dies due to weaning costs the producer money.

THE STRESS RESPONSE

The stress response, as defined by Dohms and Metz (1991), is an adaptive response by an animal to disruptions to homeostasis. These disruptions could be from any external or internal stimulus, whether physical, psychological, or social. As stated previously, there are an abundance of stressors that may elicit a stress response early in a weanling pig's life. Cortisol and catecholamine concentrations can be measured and used as indicators of stress (de Groot et al., 2001). Stressors (i.e. weaning) induce an increase in the circulating cortisol concentrations through activating the hypothalamic-pituitary-adrenal (**HPA**) axis (Kusnecov and Rossi-George, 2002). Initially, the neurons that converge in the paraventricular hypothalamus are stimulated by an agonist to release corticotrophin-releasing hormone (**CRH**), which is transported to the anterior lobe of the pituitary gland by way of the hypophyseal portal, stimulating the release of adrenocorticotropic hormone (**ACTH**) from corticotropic cells (Morimoto et al., 1991). Upon its release, ACTH is transported via the circulation where it binds to adrenal cortical cell surface receptors. When bound the cells are stimulated to biosynthesize and release glucocorticoids (i.e. cortisol) from the zona fasciculate (Salak-Johnson and McGlone, 2007). Increased cortisol concentrations in weaned pigs can cause reduced immune function (suppression of leukocytes to mitogens, reduced natural killer cell activity, and neutrophilic chemotaxis), growth retardation, and excessive inflammation (Liu, 2015). Cortisol has the capability to act as a suppressant on cytokines tumor necrosis factor alpha (**TNF-α**), interferon gamma (**INF-γ**), and IL-2 by acting through cytoplasmic and nuclear receptors on antigen presenting cells (**APC**) to suppress the production of Th1 type responses (Elenkov and Chrousos, 2002). The TH1 type response is marked by the production of proinflammatory cytokines (Berger, 2000).The stress response can

also increase concentrations of globulin, acute phase proteins (haptoglobin, serum amyloid A (**SAA**), and C-reactive protein, and reduce weigh gain (Hicks et al., 1998; de Groot et al., 2001).

During weaning, when pigs are subjected to large amounts of stressors in a short period of time, the pigs may not be able to overcome the physiological effects mentioned above. Weanling pigs do not have a fully formed intestinal barrier and when combined with high amounts of stress-induced glucocorticoids, the barrier losses its ability to uptake nutrients and block invading pathogens. Increased susceptibility to pathogens in combination with the fighting that occurs due to mixing pigs from different litters leads to uncontrolled inflammation which is characterized by increased concentrations of glucocorticoids Much of this can lead newly weaned pigs with an impaired immune system, reduced weight gain and even death (Gabler et al., 2007).

Corticosteroid-binding globulin (**CBG**) controls circulating cortisol concentrations and bioavailability. The liver hepatocytes synthesize a serine protease inhibitor, CBG, which can be influenced by age, physiological conditions, and stress (Boyle et al., 2006). Circulating concentrations of CBG affect the bioavailability of cortisol, approximately 60-90% of total cortisol is bound to CBG and 10% to albumin in swine (Heo et al., 2005). The cytokine tumor necrosis factor alpha activates neutrophils to release a specific serine protease, neutrophil elastase, during inflammatory conditions. Free cortisol index (FCI) is calculated based on the serum total cortisol/CBG ratio (Adcock et al., 2007). The free cortisol index more accurately represents the activity of the HPA axis than total serum cortisol (Le Roux et al., 2003).

Gender differences play a role in the activation of the HPA axis. Administration of low-dose lipopolysaccharide (**LPS**) to pigs has been shown to increase plasma concentrations of the

cytokine IL-1β, TNF-α, and cortisol, with intact males having lower cortisol concentrations than females (Llamas Moya et al., 2006). Females were reported to have a more responsive HPA axis, as shown by a reduction of pro-inflammatory cytokines IL-6, Il-1β, and TNF-α. Results from Da Silva (1999) showed lower testosterone concentrations in rats during the first few weeks post-partum if testes were removed. The interaction between the HPA axis, the hypothalamus-pituitary-gonadal (HPG) axis and the immune system may account for these gender differences (Moya et al., 2006).

INFLAMMATION

Inflammation is characterized as a physiological response to a stimuli such as infection or injury (Calder, 2006). The body responds to the stimuli by increasing blood flow and permeability of capillaries to molecules. This allows larger molecules, 30-176 kD, like cytokines, complement, and proteins (i.e.antibodies, CBG, albumin), to cross over into the blood stream (Calder, 2006). Two physiological responses are associated with acute inflammation (Baumann and Gauldie, 1994). First, the hypothalamus alters the body temperature set point and generates a febrile response or fever. The increase in body temperature creates a less favorable environment for viruses and bacteria. Secondly, there are alterations in metabolism and gene regulation in the liver. These alteration in metabolism and gene regulation affect the production of acute phase proteins (**APP**) and CBG in the liver.

The cytokines IL-1-β, IL-6, and TNF-α assume a large role in the acute inflammatory state. Cytokines are polypeptide signaling molecules released by certain immune cells (e.g monocytes and macrophages), which can be utilized for cell-to-cell communication (Kushner, 1993). Inflammatory cytokines can be divided into 4 groups that include the following: IL-6-type

[IL-6, IL-11, leukemia inhibitory factor , oncostatin M, and ciliary neurotrophic factor]; IL-1-type (IL-1α, IL-1β, TNF-α, and TNF-β); glucocorticoids and growth factors (insulin, hepatocyte growth factor, fibroblast growth factor and TGF-β) (Baumann and Gauldie, 1994). Interleukin-1, IL-6 and TNF-α are among the first cytokines released by monocytes and macrophages during periods of acute stress and immune regulation (Changhua et al., 2005). Over secretion of these mediating cytokines can be harmful and cause hyper-inflammation, which is seen in many inflammatory diseases. The cytokines IL-1, IL-6, and TNF-α affect glucocorticoid production by binding to receptors in the hypothalamus as well as IL-1 receptor agonist on the vagus nerve to increase cortisol production in the adrenal medulla. The release of glucocorticoids into the bloodstream acts as a negative feedback mechanism by decreasing mRNA concentrations, decreasing transcription and increasing destabilization of genes. Glucocorticoids also block post-transcriptional synthesis via cAMP, and inhibiting release into the extracellular fluid thus decreasing the presence of inflammatory cytokines (Goshen and Yirmiya, 2009). Responses of glucocorticoids produce different results when released locally vs systemically. They may act to increase the production of TGF-β, an anti-inflammatory cytokine, in TH1 cell types and suppress production in glial cells under chronic stress conditions. Mild stress, such as a mild unpredictable foot shock in rats, has been seen to induce the upregulation of the cytokines IL-1β and TNF-α in alveolar macrophages. While glucocorticoids act to negatively control inflammation, catecholamines positively affect inflammation, immune response, acute phase proteins, and hematopoiesis. (Elenkov and Chrousos, 2002).

Cytokines IL-1-β and TNF-α induce the production of E-selectins (CD62E antigen-like family), intercellular adhesion molecules-1 (**ICAM-1**), and vascular adhesion molecules-1

(**VCAM-1**) (Buczynski et al., 2009). Increased capillary permeability and the increased presence of adhesion molecules allows for diapedesis of granulocytes initially, but then gradually monocytes, macrophages and lymphocytes make their way to the site of inflammation as well. Immune cells, such as these, aid in tissue repair and removal of pathogens (Kushner, 1993). Gram-negative pathogens contain endotoxin or LPS in the cell wall, which once enveloped by the present cells, incites the production of a variety of cytokines (Calder, 2006). The cytokine IL-6 is considered a major mediator in the acute phase response based upon the number of plasma proteins it affects and elevated serum concentrations in inflamed states (Kushner, 1993).

Acute phase proteins, such as haptoglobin, serum amyloid A, and C-reactive protein are increased by the presence of IL-1-β and TNF-α. The presence of IL-6 increases the production of APP, SAA, and CRP when IL-1 and TNF-α are present (Slavich and Irwin, 2014). Glucocorticoids are synergistic with IL-1 and IL-6 and act to increase the amount of receptors on the APP cell surface (Baumann and Gauldie, 1994). Not only does IL-6 serve as the major mediator affecting plasma protein synthesis, but it also has been correlated with acute phase protein changes in numerous inflammatory states (Kushner, 1993). The APP produced induce carbohydrate dyshomeostasis, oxygen radical scavengers, protease inhibitors, coagulation factors, and opsonins. Increased IL-1 induced increase in APP functions in a protective capacity in response to inflammation, specifically when stimulated by bacterial infection (Vogels et al., 1993).

Inflammation is a normal response and can be controlled through various mechanisms. When inflammation becomes uncontrolled or unnecessary there are key signs, such as hyper-expression of endothelial and leukocyte adhesion molecules, sequestration of leukocytes to sites

where they are not usually found, production of inflammatory mediators and damage to host tissues (Calder, 2006). Glucocorticoids decrease the inflammatory response by way of a negative feedback loop, which reduces the production of inflammatory cytokines. Under circumstances characterized by glucocorticoid insensitivity, which results in a reduced response to the presence of glucocorticoids, inflammation may go unchecked. Uncontrolled inflammation is allowed when the organism is under sustained acute stress from physical danger or social stress, not unlike weaning conditions (Slavich and Irwin, 2014). This is possibly due to atrophy of neurons in the prefrontal cortex and hippocampus which mimics the effect of chronic stress (Duman and Aghajanian, 2012). Associated with increased inflammation comes increased concentrations of IL-1, IL-6 and TNF-α. These increased concentrations of inflammatory cytokines have been linked to endotoxic shock, adult respiratory distress response and chronic inflammatory diseases (Emptoz-Bonneton et al., 2011). Chronic exposure to these cytokines, in particular IL-1 and TNF-α, can account for muscle loss and bone mass reduction (Baumann and Gauldie, 1994; Calder, 2006).

POLYUNSATURATED FATTY ACIDS

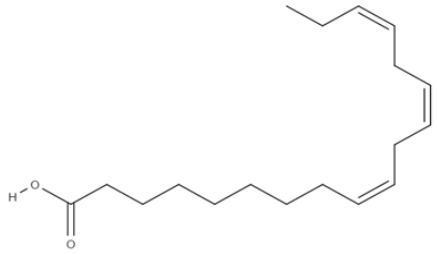
Polyunsaturated fatty acids, or PUFA, are fatty acids with two or more double bonds in their carbon backbone (Sinclair et al., 2002). There are many PUFA subtypes, but the focus here will be restricted to methylene-interrupted polyenes, specifically omega-3 (**n-3**) and omega-6 (**n-6**) PUFA. These fatty acids contain two or more *cis* double bonds and are defined by the position of the first double bond relative to the methyl end of the molecule. They comprise another broader category of PUFA denoted as essential fatty acids (**EFA**). As with essential amino acids, they are required by the body to function properly, but must be ingested by humans and other

animals as they cannot be synthesized naturally. The EFA are used by the body to produce many forms of oxidative species. To name a few: prostaglandins, lipoxins and leukotrienes, which all comprise a larger family named the eicosanoids. The PUFA are also important in membrane fluidity, as well as cell signaling (Neuringer et al., 1988; Abayasekara and Wathes, 1999).

The common practice in the swine industry is to feed diets comprised of corn and other brewers grains rich in n-6 PUFA, which effectively lower the n-3 PUFA concentration available for reduction and oxidation (Simopoulos, 1991). The U.S. Pork Center website's national swine nutrition guide shows no current recommendation or requirement for any n-3 PUFA for a gestating or lactating sow's diet. Current diets may be cost effective in the short term for finishing pigs for market, but may be deleterious to the health and well-being of the pig at time of weaning and the days that follow (Carroll et al., 1998; Hay et al., 2001).

Linoleic acid (**LA;18:2n-6**) and alpha-linolenic acid (**ALA;18:3n-3**) are EFAs determined by having 18 carbon chains (Fig 1.). Linoleic acid has two double bonds and ALA has three with both having one of those double bonds located six and three carbons away from the methyl terminus, respectively (Fig 1.). These two PUFA serve mostly as precursor molecules for long chain polyunsaturated fatty acids (Leaf, 1996). In nature, LA can be synthesized in seeds except coconut, cocoa, and palm while ALA can be found in the chloroplast of green leafy vegetables synthesized from acetate (Simopoulos, 1991; Sinclair et al., 2002). Most commonly the role in mammals for EFA like LA andALA involves conversion to PUFA such as arachidonic acid (**ARA;20:4n-6**), eicosapentaenoic acid (**EPA;20:5n-3**), and docosahexaenoic acid (**DHA:22:6n-3**), which in turn contributes to growth, neural development, reproduction, and skin function (Sung Woo et al., 2007). The EFA LA and ALA cannot be synthesized de novo

Alpha-Linolenic acid 18:3n-3



Linoleic acid 18:2n-6

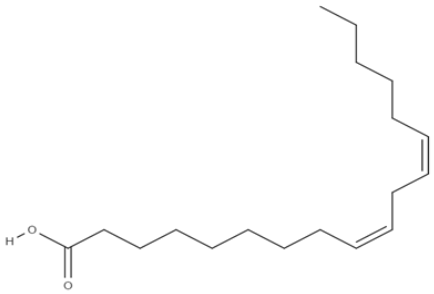


Figure 1. Structures of alpha-linolenic acid [ALA (left)] and linoleic acid [LA (right)] (obtained from molview.org).

by mammals and are desaturated with delta-5 and delta-6 desaturases. They are then lengthened to long chain PUFA (20 to 22 carbon atoms) with LA eventually yielding ARA and gamma linoleic acid (**GLA;18:3n-6**), and ALA forming EPA which then can be elongated into DHA (Makrides et al., 1995). The rate limiting step occurs when LA and ALA compete for desaturation by delta-6 desaturase as both PUFA need this desaturase to be converted to their more readily oxidized PUFAs. The PUFA ALA has a higher affinity for the desaturase and therefore can be present in smaller concentrations than LA to be effective (Leaf, 1996). As a component of the membrane phospholipids, ARA is integral in cell signaling and being converted to a majority of inflammatory eicosanoids (Innis, 2003). Mostly EPA is important for membrane fluidity and production of anti-inflammatory eicosanoid metabolites. The major PUFA in the development of the brain, retina, testis and sperm is DHA (Simopoulos, 1991). It has also been shown to be converted to D series resolvins and protectins through several pathways (Hornstra, 2000). Resolvins and protectins are a relatively newly discovered series of eicosanoid that have been shown to reduce inflammatory cytokine production in leukocytes and glial cells (Hong et al., 2003). The PUFA EPA and DHA also function to protect the cell membrane from oxidation or free radical destruction. When antioxidants are present, PUFA with the most unsaturated bonds are protected from oxidation, thus within the membrane there must be synergy between EPA and DHA, their parent phospholipids, and the antioxidants to provide the structure for a healthy membrane (Kidd, 2007).

INCORPORATION OF PUFA INTO THE SOW AND HER OFFSPRING

Lactation is the most energetically demanding phase of reproduction for a sow, requiring large quantities of nutrients and energy to produce enough milk to provide for her litter.

Approximately 35% of energy uptake is due to unbound or free fatty acids with about 8g/d in uptake for 10 L of milk/d (Farmer, 2015). Supplementation during gestation and through lactation with various oils, from linseed oil to fish oil, in an attempt to alter sow and piglet PUFA ratios. The effects of these oils have been shown to have beneficial properties associated with stress and inflammation with varied results in alteration of the sow and piglet n-6:n-3 PUFA ratio (Makrides et al., 1995; Kitajka et al., 2002; Gessner et al., 2015). The question arises when determining whether to supplement with DHA and EPA directly or their precursor ALA. From birth ARA and DHA are selectively taken up across the placental membrane and incorporated into tissues (Crawford, 2000). With ALA and not DHA being combined with cholesterol or glycerol to form cholesterol esters utilized by the brain (Sinclair et al., 2002). Crawford (2000) states, "there is little conversion of the parent essential PUFA to ARA and DHA". Conversion of ALA is also limited due to amounts of LA and other precursor PUFA intermediates that precede DHA and EPA. It has been shown that LA and ALA are transferred and converted less efficiently transplacentally than either ARA or DHA directly. A study using isotopes to measure concentrations of LA, ALA, DHA, and ARA that passed through the placenta concluded that minimal to none of the LA and ALA passed the barrier while DHA and ARA was readily incorporated into the fetus (Leaf, 1996). The placental transfer of PUFA involves membrane and cytosolic PUFA binding proteins which favor n-6 and n-3 PUFA (mainly ARA and DHA) over non-essential PUFA like LA and ALA (Rooke et al., 1999; Innis, 2005). This fact is reiterated when observing higher concentrations of ALA and LA in the dam than in the umbilical cord. Umbilical cord concentrations of DHA and EPA have been shown to be higher than in the dam (Crawford, 2000; Rooke et al., 2001). The fetal liver is the major organ for the conversion of LA

and ALA to ARA and DHA but has low functionality before birth. This would suggest that even if LA and ALA had higher placental passage rates they would not be converted in enough quantities to effect the PUFA ratio. Together, the lack of placental transfer of ALA and conversion in the fetal liver shows that feeding fish oil and other direct sources of DHA and EPA are more effective in incorporating DHA and EPA and reducing the n-6:n-3 PUFA ratio than feeding substances containing high amounts of their precursor ALA (McNeil et al., 2005).

Studies have shown that direct feed supplementation with fish oil can alter the n-6:n-3 PUFA ratio in the colostrum and milk of sows. Time of feeding before farrowing is not as limiting a factor, as transfer of PUFA to the litter has been noted even when feeding one week before expected farrowing date (Tanghe et al., 2015). As previously stated, once in the sow the concentrations of DHA and EPA are incorporated into the pig through the umbilicus, colostrum and milk. Pigs suckling from sows fed a diet during late gestation and lactation containing 3.5% and 7% menhaden fish oil compared to those fed lard had a lower overall n-6:n-3 ratio and in particular had higher concentrations of EPA than ARA in the serum, liver, and immune tissues. The overall concentration of EPA in the milk was 3%, indicating that even relatively low amounts of n-3 PUFA can alter the PUFA ratio (Fritsche et al., 1993).

PUFA AND INFLAMMATION

Since Burr and Burr (1930) first described the need for essential fatty acids (EFA's), these acids and their metabolites have been a point of interest in many animal studies determining their roles in weight gain, depression, stress and inflammation (Leat, 1962; Ferraz et al., 2011). As reviewed by Calder (2006), PUFA play a key role in the inflammatory response, particularly the n-6 PUFA ARA which comprises 20% of the n-6 PUFA present in the diet while

di-homo-γ-linoleic acid (a typically less inflammatory PUFA) only makes up 2%. This makes ARA the prime candidate for oxidation through the cyclooxygenase pathway (**COX**), particularity COX-2, and the lipoxygenase pathway (**LOX**), mainly 5-LOX. Upon cleavage from the lipid membrane by phospholipase A or C, the majority being A, and oxidation through COX-2, ARA is converted to several inflammatory series of eicosanoids, the 2 series prostaglandins, 2 series thromboxane (**TX**), leukotrienes, and hydroxyeicosatetraenoic acids. Among many effects, the eicosanoid products can induce fever, vasodilation, vascular permeability and increased production of acute phase cytokines. In particular PGE_2 increases IL-6 while LTB_4 has been shown to increase TNF-α, IL-1β, and IL-6 (Calder, 2006).

Prostaglandins are inflammatory eicosanoids characterized by the precursors from which they are formed, namely eicosanoic PUFA (C_{20}). Prostaglandins are a class of eicosanoid that have been implicated in reproductive function, platelet aggregation, kidney function, inflammation and immune response, hormone secretion, and cell signaling (Abayasekara and Wathes, 1999). An increase in the n-6:n-3 ratio functions to prolong the inflammatory response and offset homeostasis (Kidd, 2007). Prostaglandins, like PGE_2, are synthesized in the brain during an inflammatory response and its genetic expression is upregulated by the presence of IL-1β. Once PGE_2 enters the hypothalamus, which contains several different G coupled receptor subtypes specific to PGE_2, it acts to upregulate the HPA axis by stimulating the release of ACTH. Morimoto and others (1991) experimented with rats to observe the interaction between inflammation, prostaglandin synthesis, and stress concentrations. They found that prostaglandins partly mediate the stress response. Typically inflammatory conditions are characterized by a rise in temperature and ACTH concentrations. When prostaglandin synthesis (**PGE_2**) was blocked, a

rise in body temperature was halved and ACTH was reduced when compared to the control. This implies that prostaglandins play a role in the activation of the HPA axis under inflammatory conditions (Morimoto et al., 1991).

The n-3 PUFA are known to reduce the production of PGE_2 while producing less inflammatory products like the prostaglandin 3 series, resolvins and neuroprotectins, through competitive inhibition of the COX-2 pathway (Upadhaya et al., 2015). A novel function of DHA is to produce protective metabolites called docosanoids, which have been shown to have anti-inflammatory effects (Hong et al., 2003). There are three known classes of docosanoids: docosatrienes, resolvins, and protectins. Each functions differently in the mitigation of inflammation. Protectins specifically neuroprotecctin D1 counteracts potential oxidative damage to DNA in pigment epithelium cells. Resolvins function resolve inflammation by terminating ongoing inflammatory cascades (Kidd, 2007). Supplementation with fish oil has also been seen to reduce TNF-α, IL-β, PGE_2, and TX-B2 production in immune cells as EPA concentrations in the cells membrane increased (K. Fritsche et al., 1993; Caughey et al., 1996). Fritsche et al. (1993) found that enriching the diet of the sow with n-3 in the form of fish oil alters the PUFA profile of the pigs. The reduction in n-6 and increase in n-3 PUFA thereby decreased the more inflammatory series of eicosanoid production (Yao et al., 2012). Maes et al., (2000) concluded that university students with higher n-6:n-3 ratios had higher production of inflammatory cytokines IFN-γ and TNF-α under physiological stress of an important oral exam. Inversely students with a lower n-6:n-3 ratio were observed to have lower inflammatory cytokines. These results would suggest that n-3 have a beneficial effect on decreasing inflammatory cytokines under acute stressful conditions (Maes et al., 2000).

Inflammation can be controlled by non-steroidal anti-inflammatory drugs such as aspirin, glucocorticoids, gold compounds, penicillamine and methotrexate as stated by Horrocks and Yeo (1999). These drugs act to reduce COX-2 activity and thus reduce the production of PGE_2, but take a large toll on the liver and can have unwanted side effects (Astarita et al., 2015). Thus treatment of inflammation with n-3 PUFA takes less of a toll on the recipient, effectively reducing inflammation and can provide beneficial results for coronary heart disease and neurological disorders (Horrocks and Yeo, 1999).

It is important to note that PUFA not only effect the production of inflammatory mediators through COX-2, but also effect genetic regulation of transcription factor expression in peroxisome proliferator-activated receptors (**PPARγ**) pathway as well as nuclear factor kappa beta (**NF-κβ**) (Vecchini, 2003). The PPAR-γ pathway directly regulates inflammatory process gene expression by interfering with the activation of the transcription factor NF-κβ. Activation of NF-κβ happens when the inhibitory subunit is phosphorylated which allows translocation of the dimer to the nucleus. The n-3 PUFA may also act to increase the activity of the PPAR-γ pathway (Forman et al., 1997; Calder, 2009).

SUMMARY

Pigs undergo a large amount of stress within the first 24 hours of weaning. Unfamiliar pigs in conjunction with fighting increases stress and activates the inflammatory response. The HPA axis, cortisol, and CBG mediate this response and interact with cytokines to produce physiological challenges to the pig, which, if not overcome, can have deleterious effects. However n-3 PUFA play a key role in these processes and can be altered to provide physiological stability in the postweaned pig. Leading to the hypothesis, that feeding sows n-3

PUFA, in the form of protected fish oil, in late gestation and throughout lactation will reduce the stress and inflammation levels of weaned pigs as well as promote growth. The objective of exp. 1 was to determine the PFO concentration (0, 0.25, 0.5, or 1%), which, when supplied in the diet to sows during late gestation and throughout lactation, would reduce the colostrum and milk n-6:n-3 PUFA ratio most effectively. The objective of exp. 2 was to assess indicators of stress and growth during a 3 d period immediately postweaning. Weight was used an indicator of growth and pigs were weight on day of weaning (d 0) and d 3 postweaning. We used blood samples taken on d 0, 1, and 3 postweaning to determine plasma cortisol, plasma pCBG, cytokine, haptoglobin, WBC and RBC counts, hematocrit, plasma PUFA ratio, and WBC differential. The plasma cortisol and CBG concentrations were used to quantify stress through the calculation of a FCI. Cytokines (IL-6, IL-1β, and TNF-α) were analyzed using LPS challenged white blood cells in an *ex vivo* whole blood assay and used as markers for the inflammatory response. Haptoglobin concentrations were used to assess health conditions associated with inflammation. Hematocrit and RBC counts were used to determine health status. Differential and WBC counts were used to assess the immune function in the pigs.

CHAPTER 3. EFFECT OF OMEGA-3 POLYUNSATURATED FATTY ACID (N-3 PUFA) SUPPLEMENTATION TO LACTATING SOWS ON GROWTH AND INDICATORS OF STRESS IN THE POSTWEANED PIG

ABSTRACT

The first experiment was designed to determine the concentration of protected fish oil product (**PFO**), as Gromega™, to be added that would sufficiently decrease the polyunsaturated fatty acid (**PUFA**) ratio in the sows' milk and colostrum. Of the 3 diets tested (0.25%, 0.5% and 1%) with a control (0% PFO), only the 1% PFO diet had an effect on the PUFA concentrations. The docosahexaenoic acid (**DHA**) concentration tended ($P = 0.05$) to be greater in the 1% PFO diet for both colostrum and milk samples. A second experiment was designed to examine the effects of feeding the 1 versus 0% PFO supplemented diet to sows on growth, markers of acute inflammation and stress in their offspring (16 piglets/treatment group) on d 0 (day of weaning) and d 1 and 3 postweaning. Piglets from sows supplemented the 1% PFO diet had greater gains in weight ($P = 0.03$) postweaning. These pigs also had a lower ($P < 0.01$) n-6:n-3 PUFA in the plasma when compared to piglets on the control diet. There was an overall treatment effect ($P = 0.02$) on plasma total cortisol, observed by lower concentrations in pigs on the 1% PFO diet. Plasma corticosteroid-binding globulin (**CBG**) concentrations were not different between treatment groups but were lower ($P < 0.001$) on d 1 and 3 when compared to d 0. The calculated free cortisol index [**FCI** (cortisol/CBG)] of pigs on the 1% diet was lower ($P = 0.02$) on d 1 and 3 when compared to the controls. The cytokines, IL-1β [beta], IL-6, and TNF-α [alpha] were measured following an ex vivo lipopolysaccharide (**LPS**) stimulation of monocytes and neutrophils in whole blood collected on d 0 and 1. Pigs on the 1% PFO diet tended to have a lower ($P = 0.098$) mean concentration of TNF-α in response to LPS when compared with that of the controls. These results suggest that providing a PFO supplement as 1% of the diet to sows

beginning in late gestation and during lactation can lower the n-6:n-3 PUFA ratio in their offspring, which may reduce the acute physiological stress response in the pigs postweaning.

INTRODUCTION

Pigs undergo a large amount of stress within the first 24 hours of weaning. Unfamiliar pigs in conjunction with fighting increases stress and activates the inflammatory response (Campbell et al., 2013). The hypothalamic-pituitary-adrenal (**HPA**) axis, cortisol, and corticosteroid binding globulin (**CBG**) mediate this response and interact with cytokines to produce physiological challenges to the pig, which, if not overcome, can have deleterious effects (Elenkov and Chrousos, 2002; Goshen and Yirmiya, 2009). However omega-3 (**n-3)** polyunsaturated fatty acids (**PUFA**) play a key role in these processes and can be altered to provide physiological stability (Calder, 2006). Studies in which humans and rats are fed dietary n-3 PUFA have reduced n-6:n-3 PUFA ratio and show reductions in glucocorticoid concentrations when stressed (Hamazaki et al., 1999; Borsonelo et al., 2011). Supplementation with n-3 PUFA has also been shown to reduce the production of acute phase cytokines, IL-6, IL-1β, and tumor necrosis factor α (**TNF-α**) after lipopolysaccharide (**LPS**) challenge in pigs (Liu et al., 2003; Upadhaya et al., 2015). There are many sources of n-3 PUFA that have shown capable of reducing the n-6:n-3 PUFA ratio in sow milk and piglet tissues (Fritsche et al., 1993; Smit et al., 2013). In a study conducted by Gabler et al. (2007), 3 sources of n-3 PUFA: protected fish oil (**PFO**) as Gromega^{TM}, DHA from Schizochytrium algae, and dried coconut fat were fed to sows during late gestation and into lactation. Milk samples from sows supplemented the PFO at 1.5% showed the greatest reduction in n-6:n-3 PUFA ratio resulted.

Therefore, the hypothesis of this study was that feeding sows n-3 PUFA, in the form of a PFO in late gestation and throughout lactation will reduce the stress and inflammation levels of weaned pigs as well as promote growth upon weaning. The first objective of this study was to determine the PFO concentration (0, 0.25, 0.5, or 1%), which, when supplied in the diet to sows during late gestation and throughout lactation, would significantly reduce the colostrum and milk n-6:n-3 PUFA ratio. The second objective was to assess indicators of stress and growth in pigs postweaning from sows fed a diet supplemented with the percentage of PFO determined from our initial study that significantly reduced the n-6:n-3 PUFA ratio.

MATERIALS AND METHODS

Ethics Statement

The following experiments were conducted at the University of Kentucky swine facility (Versailles, KY) and University of Tennessee Johnson Animal Research and Teaching Unit (**JARTU**; Knoxville, TN). Animal use and sample collection procedures used in this study were pre-approved by the University of Tennessee Animal Care and Use Committee.

Experimental Design, Animals, Housing, and Diets

Exp. 1

A preliminary experiment conducted in the fall of 2014 used 18 time-bred gilts (*n*=14 [Yorkshire x Landrace x Duroc] and *n*=4 [Yorkshire x Landrace]) weighing 189 ± 11.58 kg from the University of Kentucky (UK) swine herd to determine sufficient dietary concentrations of fish oil (**PFO**) product (Gromega™, JBS United, Inc., Sheridan, IN) that would significantly alter the colostrum and milk n-6:n-3 PUFA ratio. The Gromega™ supplement contained 39.2% fat (by acid hydrolysis) with EPA and DHA making up 13.8 and 11.4% of the total fat

respectively. Gilts were artificially inseminated with purchased semen collected from Krskopolje, Duroc, or Bulgarian White breeds (Swine Genetics International [SGI], Cambridge, IA). Gilts were subject to a completely randomized design (**CRD**) and selected to receive a gestation and lactation diet supplemented with 0 (n = 5), 0.25 (n = 4), 0.5 (n = 4) or 1% (n = 5) added PFO from 101 ± 2 d of gestation to d 16 of lactation (Table 1 and 2). All diets were formulated using National Research Council (NRC, 1998) requirements for gestating and lactating sows. Gilts were fed 6.36 kg/d of the gestation diet up to the day of farrowing. Beginning on day of farrowing, sows were fed 5.45 kg/d of the lactation diet and if fully consumed, 0.91 kg/d of feed was added up to a maximum 9.09 kg/d. On 104 ± 2 d of gestation, 8 of the 18 bred gilts were transported from UK to JARTU animal facility farrowing room and housed in individual farrowing crates. Farrowing room temperature was thermostatically maintained at 23°C. All gilts were allowed unlimited access to water through nipple waterers and limit fed using conventional dry feeders with adjustment plates until farrowing. Gilts farrowed within 48 h of each other with a litter number ranging between 7 to 15 piglets averaging1.46 ± 0.51 kg. Piglets were provided supplemental heat via heating pads and processed within 3 d of birth. Processing consisted of spraying the naval cords with Betadine solution (Purdue Products L.P., Stamford, CT), administering 1 mL of iron dextran intramuscularly (INFeD; ACTAVIS, Parsippany, NJ), clipping needle teeth, ear notching and tagging, tail docking, and castration of the males. Pigs were allowed free access to water and any feed remaining in the sow's feeder.

Exp. 2

In fall of 2015, a total of 8 time-bred gilts (n=1 Yorkshire and n=7 Yorkshire x Landrace)

Table 1. Percentage composition of the gestation diet fed to gilts from late gestation up to farrowing (as-fed basis)

	Percent fish oil product			
Ingredient, %	0%	0.25%	0.50%	1%
Yellow corn, ground	83.12	82.90	82.67	82.22
Dehulled soybean meal, 48% CP	10.05	10.03	10.00	9.95
Alfalfa Meal	2.50	2.50	2.50	2.50
Choice white grease	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.55	1.55	1.55	1.55
Limestone (Ground)	0.83	0.83	0.83	0.83
Salt, (plain)	0.50	0.50	0.50	0.50
Vitamin mix[1]	0.10	0.10	0.10	0.10
Trace mineral premix[2]	0.05	0.05	0.05	0.05
Choline Mix, 50%	0.10	0.10	0.10	0.10
Santoquin[3]	0.20	0.20	0.20	0.20
Gromega, protected fish oil	0.00	0.25	0.50	1.00
Calculated composition				
ME content of diet (kcal/kg)	3,303	3,303	3,303	3,303
CP, %	12.11	12.11	12.11	12.11
Lysine, %	0.54	0.54	0.54	0.54
Calcium, %	0.75	0.75	0.75	0.75
Phosphorous, %	0.60	0.60	0.60	0.60

[1]Supplied per kilogram of diet: 6,600 IU vitamin A, 1,320 IU vitamin D_3, 66 IU vitamin E, 6.6 mg vitamin K (menadione sodium bisulfate complex), 8.8 mg riboflavin, 22 mg d-pantothenic acid, 88 mg niacin, 6.6 mg vitamin B_6, 33 µg vitamin B_{12}, 220 µg d-biotin, and 1,320 µg folic acid.

[2]Supplied per kilogram of diet: 100 mg Zn as ZnO, 120 mg Fe as $FeSO_4 \cdot H_2O$, 45 mg Mn as MnO, 12 mg Cu as $CuSO_4 \cdot 5H_2O$, 1.5 mg I as CaI_2O_6, and 0.30 mg Se as $NaSeO_3$.

[3]The Santoquin product (Novus International Inc., St. Louis, MO) supplied 130 mg of ethoxyquin per kilogram of basal diet.

[4]Gromega product (JBS United, Inc., Sheridan, IN) fatty acid profile was 39.2% total fat (by acid hydrolysis) with myristic (14:0) 8.1%, myristoleic (15:0) 0.76%, palmitic (16:0) 17.08%, palmitoleic [(16:1) 11.85%, (17:0) 0.61%, (17:1) 1.65%], stearic (18:0) 3.18%, elaidic (18:1n-9) 1.68%, oleic (18:1n-9) 5.46%, vaccenic (18:1n-7) 4.15%, linoleic (18:2) 1.56%, linolenic [(18:3) 1.43% and (18:4) 2.85%], arachidic [(20:0) 0.2% and (20:1n-9) 1.01%], arachidonic (20:4n-6) 1.15%, eicosapentaenoic (20:5n-3) 13.75%, docosanoic (22:0) 0.26%, erucic (22:1n-9) 0.22%, docosapentaenoic (22:5n-3) 2.46%, docosahexaenoic (22:6n-3) 11.39%, and nervonic (24:1n-9) 0.46%.

Table 2. Percentage composition of the lactation diet fed to sows immediately after farrowing up to weaning (as-fed basis)

	Percent fish oil product			
Ingredient, %	0%	0.25%	0.50%	1%
Yellow corn, ground	67.56	67.33	67.11	66.66
Dehulled soybean meal, 48% CP	25.60	25.58	25.55	25.50
Alfalfa Meal	2.50	2.50	2.50	2.50
Choice white grease	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.21	1.21	1.21	1.21
Limestone (Ground)	0.89	0.89	0.89	0.89
Salt, (plain)	0.50	0.50	0.50	0.50
Vitamin mix[1]	0.10	0.10	0.10	0.10
Trace mineral premix[2]	0.05	0.05	0.05	0.05
Choline Mix, 50%	0.10	0.10	0.10	0.10
Dynamate[3]	0.50	0.50	0.50	0.50
Santoqiun[4]	0.20	0.20	0.20	0.20
Gromega, protected fish oil[5]	0.00	0.25	0.50	1.00
Calculated composition				
ME content of diet (kcal/kg)	3,290	3,290	3,290	3,290
Crude Protein, %	18.19	18.19	18.19	18.19
Lysine, %	0.97	0.97	0.97	0.97
Calcium, %	0.75	0.75	0.75	0.75
Phosphorous, %	0.60	0.60	0.60	0.60

[1]Supplied per kilogram of diet: 6,600 IU vitamin A, 1,320 IU vitamin D_3, 66 IU vitamin E, 6.6 mg vitamin K (menadione sodium bisulfate complex), 8.8 mg riboflavin, 22 mg d-pantothenic acid, 88 mg niacin, 6.6 mg vitamin B_6, 33 μg vitamin B_{12}, 220 μg d-biotin, and 1,320 μg folic acid.

[2]Supplied per kilogram of diet: 100 mg Zn as ZnO, 120 mg Fe as $FeSO_4 \cdot H_2O$, 45 mg Mn as MnO, 12 mg Cu as $CuSO_4 \cdot 5H_2O$, 1.5 mg I as CaI_2O_6, and 0.30 mg Se as $NaSeO_3$.

[3]The Dynamate product (Mosaic Feed Ingredients, South Riverview, FL) contained per kilogram: 180 g of K, 110 g of Mg, and 220 g of S.

[4]The Santoquin product (Novus International Inc., St. Louis, MO) supplied 130 mg of ethoxyquin per kilogram of basal diet.

[5]Gromega product (JBS United, Inc., Sheridan, IN) fatty acid profile was 39.2% total fat (by acid hydrolysis) with myristic (14:0) 8.1%, myristoleic (15:0) 0.76%, palmitic (16:0) 17.08%, palmitoleic [(16:1) 11.85%, (17:0) 0.61%, (17:1) 1.65%], stearic (18:0) 3.18%, elaidic (18:1n-9) 1.68%, oleic (18:1n-9) 5.46%, vaccenic (18:1n-7) 4.15%, linoleic (18:2) 1.56%, linolenic [(18:3) 1.43% and (18:4) 2.85%], arachidic [(20:0) 0.2% and (20:1n-9) 1.01%], arachidonic (20:4n-6) 1.15%, eicosapentaenoic (20:5n-3) 13.75%, docosanoic (22:0) 0.26%, erucic (22:1n-9) 0.22%, docosapentaenoic (22:5n-3) 2.46%, docosahexaenoic (22:6n-3) 11.39%, and nervonic (24:1n-9) 0.46%.

weighing between 206.89 ± 40.89 kg were transported from the UK swine facility to the JARTU farrowing room at 105 ± 3 d of gestation. All gilts were artificially inseminated with Duroc Choice Semen purchased from SGI. Upon arrival, gilts were randomly housed in individual farrowing crates where only the control and 1% PFO diets were used as dietary treatments following the results of Exp. 1. Diets were formulated and fed as described in Exp. 1. All gilts farrowed within 48 h of each other with 8 to 14 piglets per litter and birth weight of 2.16 ± 0.81 kg. Piglets were housed and processed as in Exp. 1. Beginning 1 wk prior to weaning, piglets were provided with ad libitum access to their dam's lactation diet using creep feeders. Upon weaning (31 ± 2 d of age) 32 pigs consisting of 8 males and 8 females were selected from litters of each dietary treatment group based upon a uniform weight (6.25 ± 0.9 kg) and transferred relative to experimental diet to two nursery pens (3.05 m x 3.05 m) located within the farrowing room. Pens contained nipple cup waterers and a large self-feeder providing ad libitum access to either the control or 1% PFO nursery feed and water. The two dietary treatments were formulated based upon NRC (2012) nutrition requirements for nursery feed (Table 2). All room conditions were the same as described in Exp. 1.

Tissue and Performance Measurements

Colostrum and milk samples were taken in the same manner for Exp. 1 and 2. Samples ranged from 10 to 45 mL collected in 50 mL falcon tubes from multiple teats within 24 h of farrowing and 16 ± 2 d post-farrowing and placed in -80°C freezer until later analysis.

Blood samples (6 ± 2 mL) were collected from the 32 selected pigs in Exp. 2 via cranial vena cava puncture in 10 mL sodium heparin coated vacutainer tubes (Becton Dickinson Vacutainer Systems; Becton, Dickinson and Company, Franklin Lakes NJ) immediately prior to weaning (d

0) and d 1, and 3 postweaning. Blood samples were immediately stored on ice and processed within 1 h following collection. Pigs were weighed following blood collection on d 0 and 3. Aliquots of whole blood (1 mL) collected on d 0 and 1 were designated for use in an ex vivo lipopolysaccharide (**LPS**) cytokine assay. Aliquots of whole blood (100 µL) collected on d 0, 1, and 3 d were used to prepare blood smears and determine hematocrit. The remaining blood samples were centrifuged at 3134 x *g* for 20 min at 4°C. Plasma was pipetted into cryogenic vials and stored at -80°C until further analysis for concentrations of cortisol, porcine corticosteroid binding globulin (**pCBG**), haptoglobin, and phospholipid analysis.

Phospholipid Extraction from Colostrum, Milk, and Plasma

Colostrum, milk, and plasma (200 µL) samples were extracted for phospholipid analysis using the same method for Exp. 1 and 2 following the procedure of Xiong et. al. (2012). Samples were pipetted into 1.5 mL Eppendorf tubes and 40 µL of internal standard (acetylcholine-d13: 0.0112 g, betaine-d11: 0.0386 g, choline-d9: 0.024 g, lysophosphatidylcholine-d3: 0.005 g, phosphatidylcholine-d9: 0.0096 g, phosphocholine-d9: 0.2404 g, 31phingomyelin-d3-13C: 0.021 g) dissolved in methanol, was added to the sample. A HPLC grade extraction solvent containing chloroform, methanol, and water (1:2:0.8; 1 mL0) was added for a total of 1.24 mL of solution. Tubes were vortexed at 2.5 x *g* in 4°C for 5 min and the resulting supernatant transferred to a clear glass vial. The extraction process was repeated two more times by adding 1 mL of extraction solvent to the pellet then vortexed, centrifuged and transferred to the glass vial. Collected supernatant was dried under a steady stream of nitrogen and re-dissolved in 3 mL of methanol. Solution volumes of 300 µL were pipetted into auto sample vials for phospholipid analysis by LC-MS/MS. Intra- and inter- assay

Table 3. Percentage composition of the nursery diet fed to pigs 1 wk prior to weaning and 3 d postweaning (as-fed basis)

	Percent fish oil product	
Ingredient, %	0%	1%
Corn	49.73	48.73
Soybean meal	31.60	31.60
Fish meal	3.00	3.00
Whey dried	10.00	10.00
Grease	2.30	2.30
Corn starch	0.30	0.30
L-Lysine	0.23	0.23
DL-Methionine	0.24	0.24
L-Threonine	0.18	0.18
L-Tryptophan	0.01	0.01
Dicalcium Phosphate 1	0.76	0.76
Limestone	0.88	0.88
Salt	0.50	0.50
Sow TM4[1]	0.15	0.15
Vitamin mix[2]	0.10	0.10
Santoquin[3]	0.02	0.02
Gromega, protected fish oil[4]	0.00	1.00

Table 3. Continued

	Protected fish oil	
Calculated composition	0%	1%
SID amino acids, %		
Lys	1.35	1.35
Met	0.56	0.56
Ile	0.87	0.87
Thr	0.94	0.94
Trp	0.26	0.26
ME content of diet, kcal/kg	3,406	3,406
CP, %	22.82	22.82
Lysine, %	1.35	1.35
Calcium, %	0.80	0.80
Phosphorous, %	0.65	0.65
Available P, %	0.40	0.40

[1]Supplied per kilogram of diet: 125 mg Zn as $ZnSO_4 \cdot H_2O$, 100 mg Fe as $FeSO_4 \cdot H_2O$, 50 mg Mn as $MnSO_4 \cdot H_2O$, 20 mg Cu as $CuSO_4 \cdot 5H_2O$, 0.35 mg I as CaI_2O_6, and 0.30 mg Se as $NaSeO_3$.
[2]Supplied per kilogram of diet: 6,600 IU vitamin A, 1,320 IU vitamin D_3, 66 IU vitamin E, 6.6 mg vitamin K (menadione sodium bisulfate complex), 8.8 mg riboflavin, 22 mg d-pantothenic acid, 88 mg niacin, 6.6 mg vitamin B_6, 33 µg vitamin B_{12}, 220 µg d-biotin, and 1,320 µg folic acid.
[3]The Santoquin product (Novus International Inc., St. Louis, MO) supplied 130 mg of ethoxyquin per kilogram of basal diet.
[4]The Gromega product (JBS United Inc., Sheridan, IN) fatty acid profile was 39.2% total fat (by acid hydrolysis) with myristic (14:0) 8.1%, myristoleic (15:0) 0.76%, palmitic (16:0) 17.08%, palmitoleic [(16:1) 11.85%, (17:0) 0.61%, (17:1) 1.65%], stearic (18:0) 3.18%, elaidic (18:1n-9) 1.68%, oleic (18:1n-9) 5.46%, vaccenic (18:1n-7) 4.15%, linoleic (18:2) 1.56%, linolenic [(18:3) 1.43% and (18:4) 2.85%], arachidic [(20:0) 0.2% and (20:1n-9) 1.01%], arachidonic (20:4n-6) 1.15%, eicosapentaenoic (20:5n-3) 13.75%, docosanoic (22:0) 0.26%, erucic (22:1n-9) 0.22%, docosapentaenoic (22:5n-3) 2.46%, docosahexaenoic (22:6n-3) 11.39%, and nervonic (24:1n-9) 0.46%.

CV was 84.62% and 35.88% for the pooled unknown cow milk samples (19.23 μM/L). Milk unknowns were used for both sow colostrum and milk samples. Intra- and inter- assay CV was 53.81% and 65.6% for the unknown pooled plasma samples (0.07 μM/L).

Cortisol Analysis

Plasma cortisol samples were analyzed for total cortisol concentration by RIA (MP Biomedicals, LLC, Orangeburg, NY) as reported previously (Adcock et al. 2006). Intra- and inter-assay CV was 8.6 and 3.0% for the low control (41.79 nmol/L) and 5.4 and 8.8% for the high control (163.58 nmol/L) cortisol standards.

Corticosteroid-Binding Globulin Analysis

The plasma pCBG concentrations were determined by a direct ELISA, as previously described by Roberts *et al*. 2003. Intra- and inter-assay CV of a pooled plasma sample was 6.1 and 11.6% for pool A (35.58 mg/L) and 9.3 and 10.6% for pool B (35.42 mg/L) respectively.

Free Cortisol Index

The free cortisol index was calculated using the ratio of plasma total cortisol (nmol/L) to pCBG (mg/L) concentration (le Roux et al., 2002) and reported in units of nmoL/mg.

Haptoglobin Analysis

Porcine haptoglobin samples were analyzed using a radial immunodiffusion (**RID**) kit (Ecos Institute, Aasahi, Furukawa, Miyagi, Japan). Plasma samples 100 μL were diluted five-fold with PBS pH 7.4. Aliquots of diluted sample (5 μL) were pipetted into individual wells on agar test plates then incubated at 37°C for 24 h. During incubation a precipitin reaction occurred forming a visible ring in the gel. Measurements of sample ring diameter were performed under UV light. Ring diameter was proportional to the concentration of haptoglobin in the diluted

sample. The intra-assay CV was 9.5% for duplicate haptoglobin samples (635.1 μg/mL). The inter-assay CV was 5.4% for the low (252.04 μg/mL) and 1.5% for the high (1512 μg/mL) haptoglobin controls.

RBC, WBC, Hematocrit, and WBC Differentials

Determination of RBC and WBC counts and hematocrit was performed using a scil Vet ABC Hematology Analyzer (scil animal care company, Gurnee, IL). Blood smears were prepared for determination of WBC differentials using a PROTOCOL Hema 3 Staining System (Thermo Fisher Scientific Inc., Kalamazoo, MI). In brief, slides were submerged into 3 different staining solutions in 30 s intervals. Slides were then allowed to dry. Once dry, slides were prepared for oil immersion light microscopy and neutrophils and lymphocytes identified. A total 100 granular (neutrophil) and non-granular (lymphocytes) were counted for each slide. Recorded values were used to calculate the neutrophil to lymphocyte ratio (N:L) by dividing the amount of counted neutrophils by the amount of counted lymphocytes.

Statistical Analysis

All data was analyzed using the mixed model ANOVA GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Treatments (PFO or control) were assigned using a CRD. Treatment and sample (colostrum or milk) were fixed effects in a mixed model, tested using pig as the experimental unit. Degrees of freedom and fixed effects were adjusted using the Kenward-Roger method. Least square means were compared and evaluated using least significant difference mean separation method with significance determined at $P \leq 0.05$. All data are represented as least square means ± SEM.

Plasma PUFA, cortisol, pCBG, haptoglobin, total WBC/RBC, hematocrit, N/L ratio and weight were analyzed utilizing a CRD with repeated measures (day) structured using AR(1) for the model. Fixed effects were treatment, sex, and day with pig as the experimental unit in a mixed model. Cytokine data was determined using a CRD with a split-split plot. Mixed model fixed effects were diet, sex, day and endotoxin (LPS or control). Due to unequal variances, a log transformation was used to estimate the mean effects of cortisol, FCI, and cytokines. Normality was not an issue (0.92) for all other effects, there were no severe outliers or influential points, and equal variance was less than a 5-fold difference. Sex was determined to be non-significant and was therefore removed from the model.

RESULTS

The n-6:n-3 PUFA ratio was calculated as total n-6 being 20:4 and 18:2 and the total n-3 being 18:3, 20:5, 22:5, 22:6. Each total was then divided by the total amount of phosphatidylcholine. Individual PUFA were calculated in the same manner (Smit et al., 2013).

Each sow within its respective dietary treatment farrowed 12 ± 2 piglets with two stillborn and one mummy overall in exp. 1. In exp. 2 sows farrowed 13 ± 2 pigs with 4 stillborn and no mummies overall. For both experiments on d 16 the only deaths were due to crushing and suffocation from the sow. In exp. 2 pigs were selected based on weight and sex. All 64 pigs were selected from each litter within a treatment group and weighed on average 7.45 ± 1.51 kg. For each dietary treatment group, 16 piglets were selected based on sex with 8 gilts and 8 barrows.

EXP. 1

Fatty Acids in the Colostrum. The greatest (P = 0.05) concentrations of DHA in the sows' colostrum tended to be when 1% PFO was added to the diet. The DHA concentration in

the colostrum of sows supplemented with 0.25% and 0.5% PFO diets did not differ from the control. The concentrations of EPA and ARA did not differ ($P = 0.36$) due to treatment (Fig. 2).

Fatty Acids in the Milk. The concentration of DHA in milk collected from sows fed the 1% PFO diet tended to be greater ($P = 0.09$) than that measured in sows fed other dietary treatments. The concentration of EPA and ARA did not differ as a result of treatment (Fig. 3).

EXP. 2

Pig Weights. Pigs from the 1% PFO treatment group weighed more ($P = 0.03$) on d 3 postweaning compared to the control pigs (Fig 4.)

Fatty Acids in the Colostrum and Milk. The n-6:n-3 PUFA ratio did not differ among treatment in colostrum or milk ($P = 0.7$) when compared to the control (Fig. 5).

Plasma Fatty Acids. There was a treatment effect ($P <0.01$) on the n-6:n-3 PUFA ratio with pigs on the 1% PFO diet having a lower ratio than the control pigs. A day effect ($P < 0.01$) was observed with d 1 having the lowest n-6:n-3 PUFA ratio than either d 1 or d 3 (Fig. 6).

Plasma Cortisol. Of plasma cortisol values across all 3 days, pigs fed the 1% diet had lower concentrations ($P = 0.02$) of plasma cortisol than the pigs on the control diet. There was an observed day effect ($P = 0.01$) with d 1 having the highest concentrations of cortisol. Cortisol concentrations, with regard to day, did not differ on d 0 when compared to d 3 (Fig. 7).

Plasma CBG Concentration. Plasma CBG concentrations did not differ ($P > 0.1$) between treatments on d 0, 1, or 3. There was an overall day of sampling effect for CBG concentration, such that the d 1 and 3 concentrations were lower ($P < 0.001$) when compared with d 0 (Fig. 8).

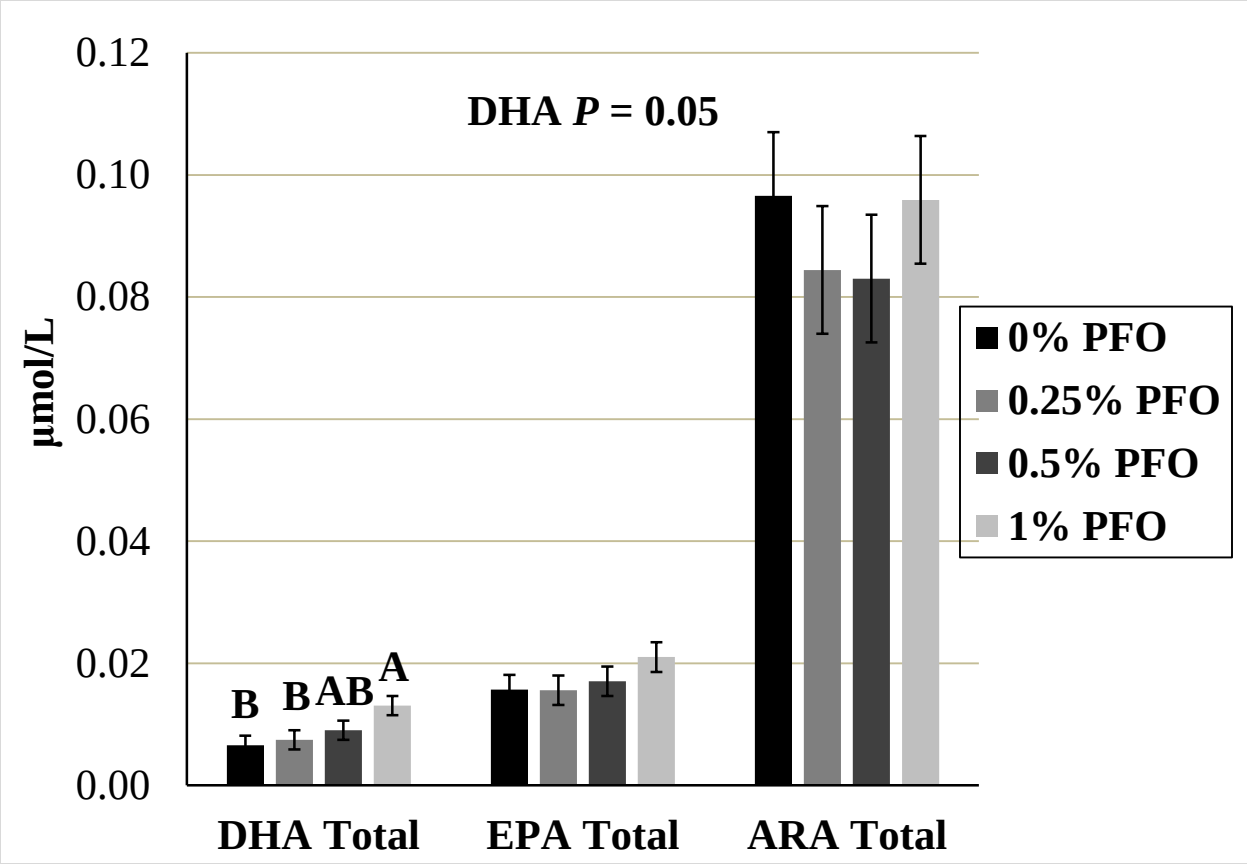


Figure 2. Colostrum concentrations of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) from sows fed a corn-soybean based diet supplemented with 0 (n = 5), 0.25 (n = 4), 0.5 (n = 4), and 1% (n = 5) PFO from 101 ± 2 d of gestation to d 16 of lactation and collected within 24 h of farrowing. Means ± SEM with different letters differ (*P* = 0.05).

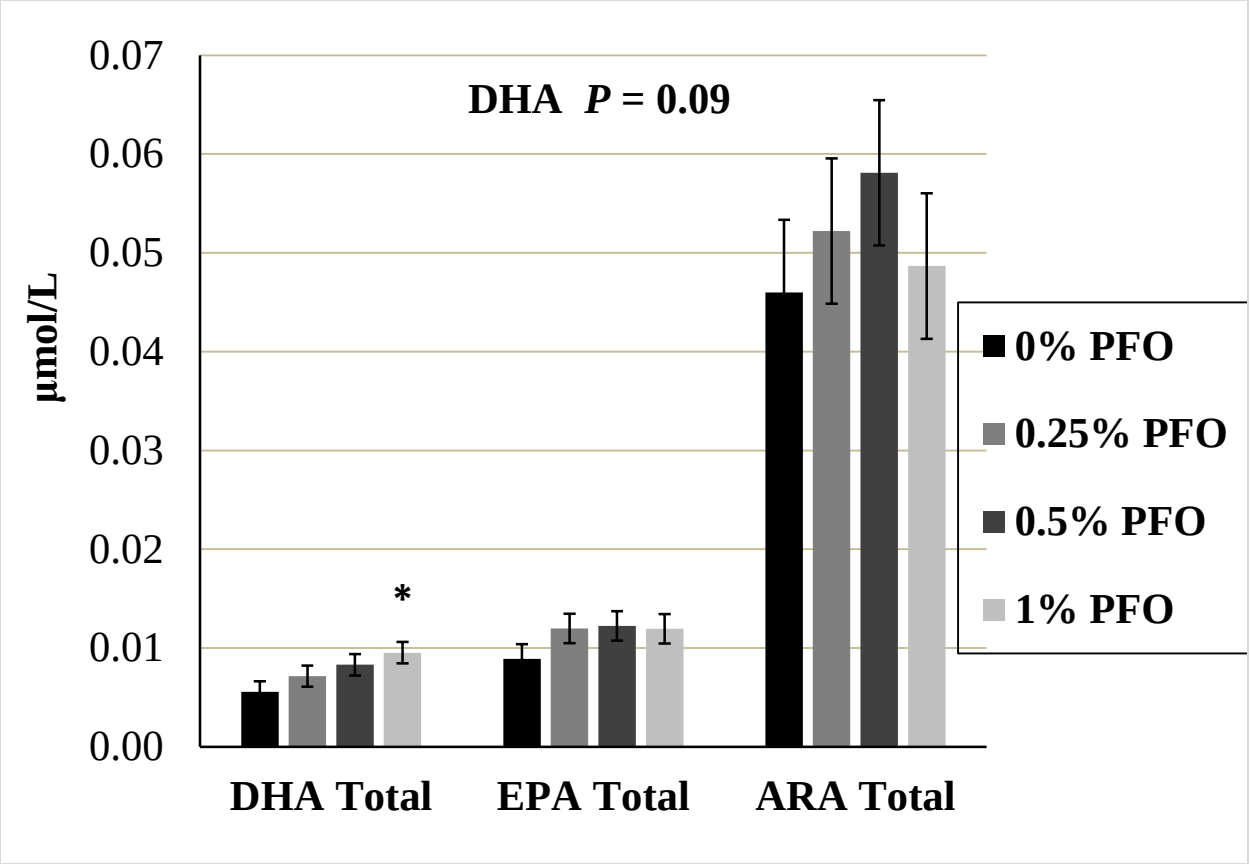


Figure 3. Milk concentrations of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) from sows fed a corn-soybean based diet supplemented with 0 (n = 5), 0.25 (n = 4), 0.5 (n = 4), and 1% (n = 5) PFO from 101 ± 2 d of gestation to 16 ± 2 d of lactation and collected on 16 ± 2 d of lactation. Means ± SEM with asterisks differ (*P* = 0.9).

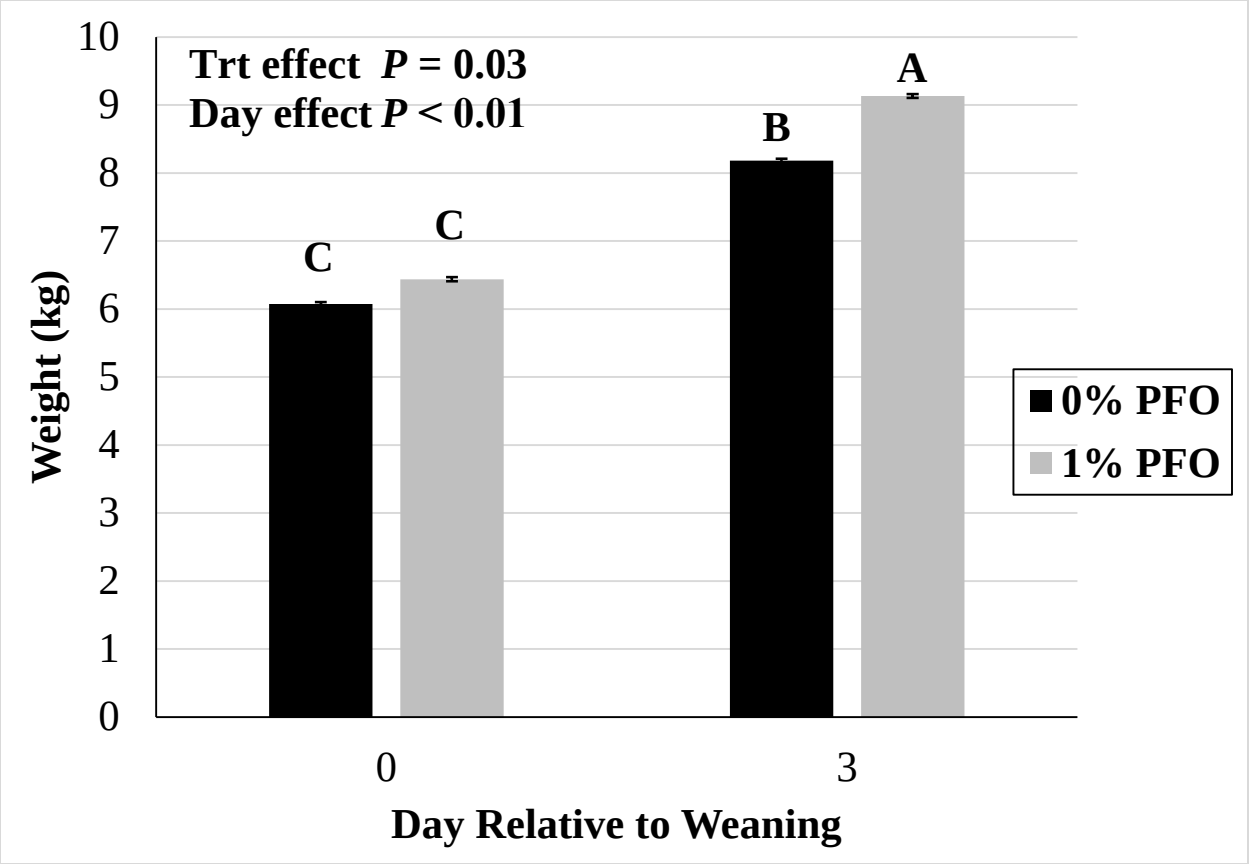


Figure 4. Change in body weight from weaning (d 0) to d 3 postweaning in pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Mean ± SEM different letters differ (*P* = 0.03) for treatment effect and (*P* < 0.01) for day effect.

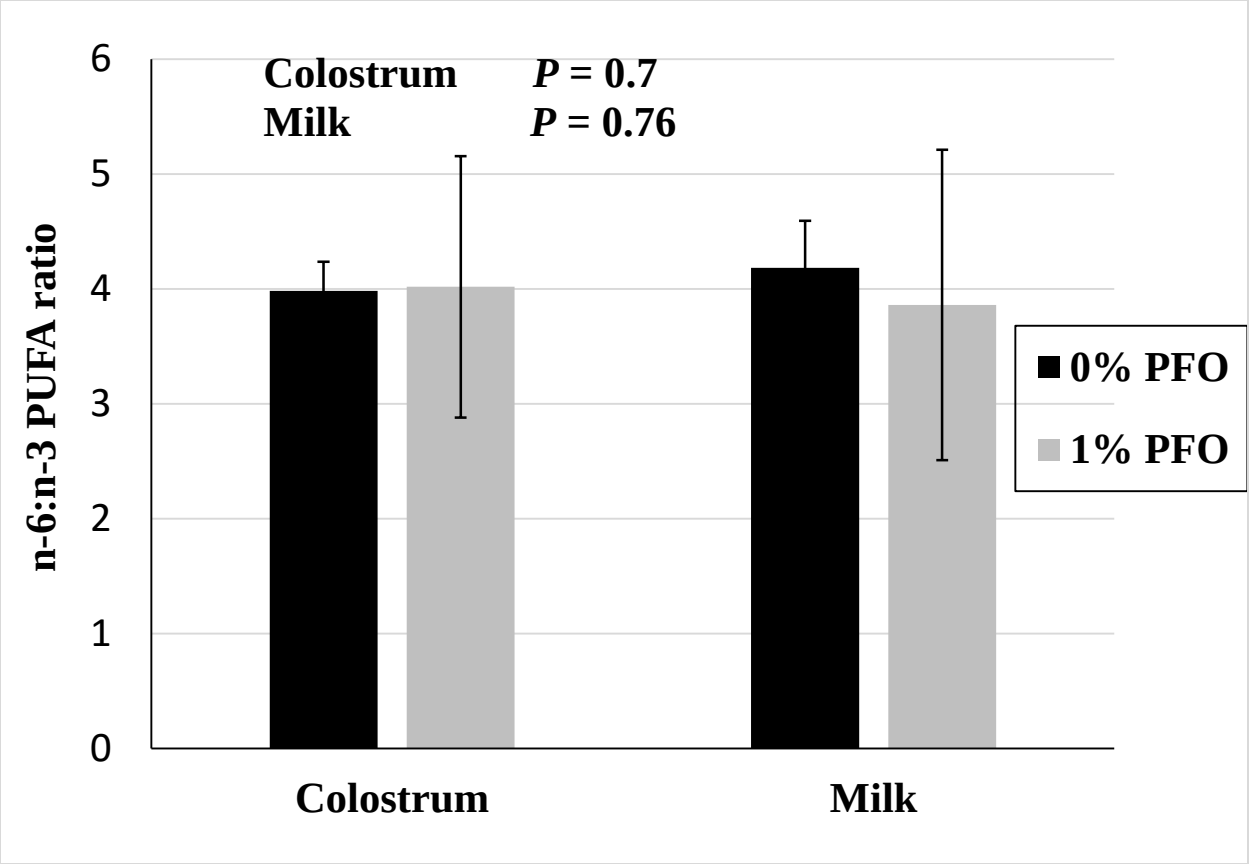


Figure 5. Sow n-6:n-3 PUFA ratio receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) from 105 ± 3 d gestation until weaning 31 ± 2 d postfarrowing. Means ± SEM with different letters differ (*P* = 0.7 and 0.76) for colostrum and milk, respectively.

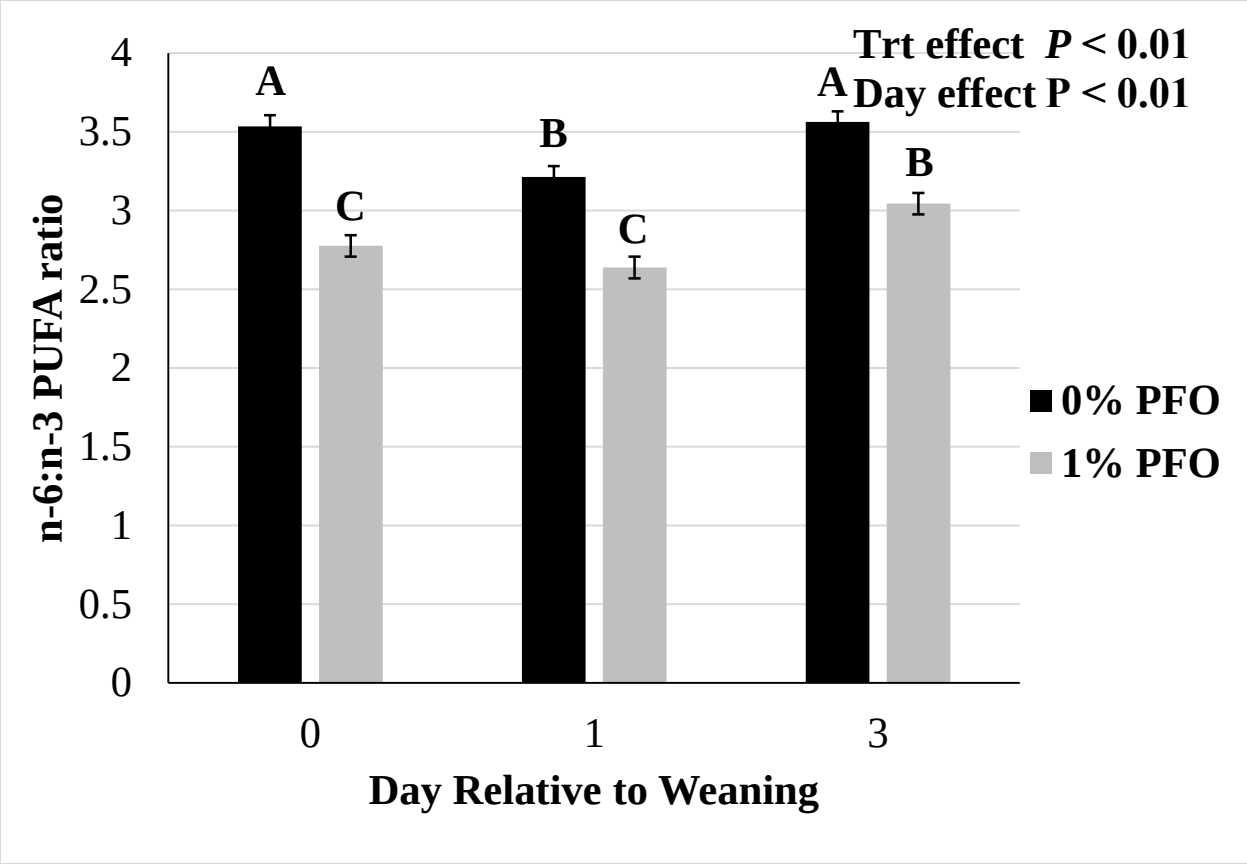


Figure 6. n-6:n-3 PUFA ratio from weaning (d 0), 1, and 3 d postweaning in pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* < 0.1) for treatment and day effect.

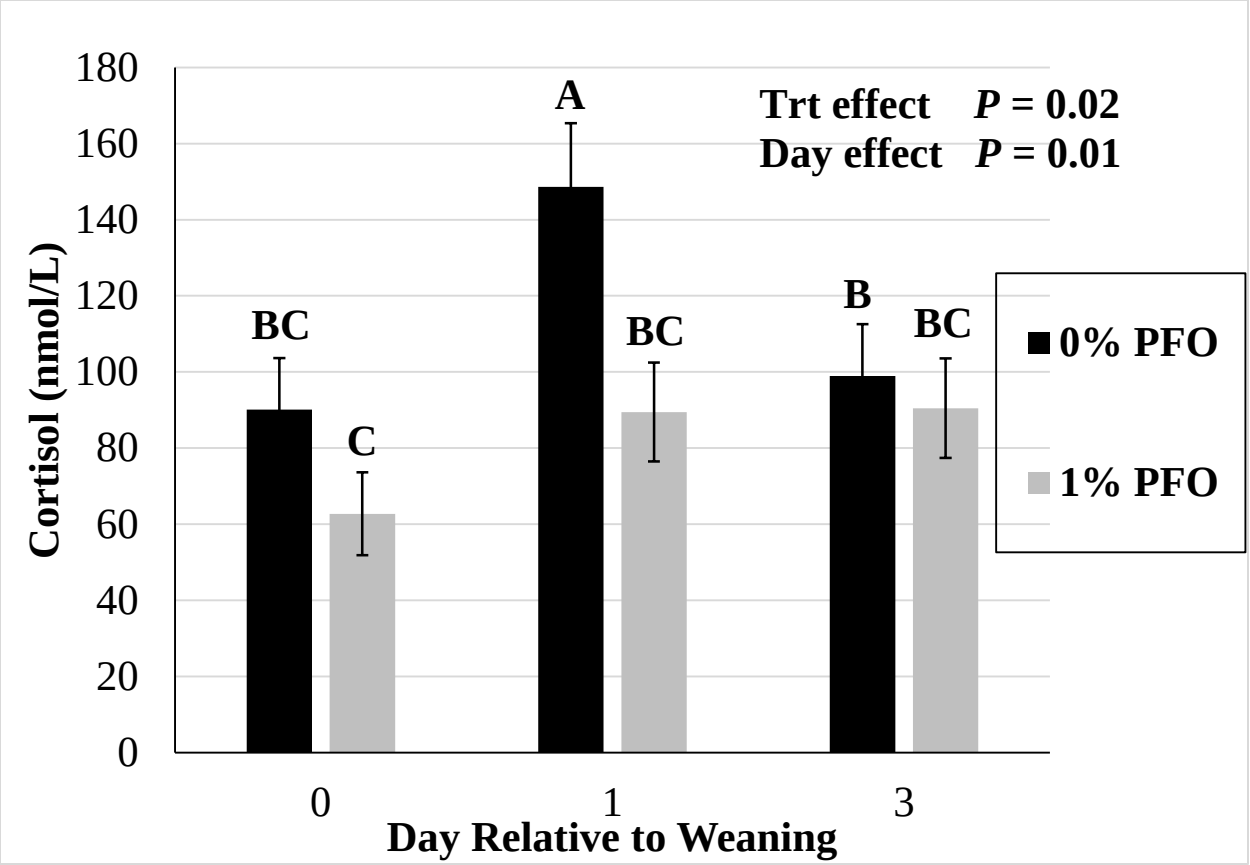


Figure 7. Plasma cortisol concentrations from weaning (d 0), 1, and 3 d postweaning in pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* = 0.02) and (*P* =0.01) for treatment and day effect, respectively.

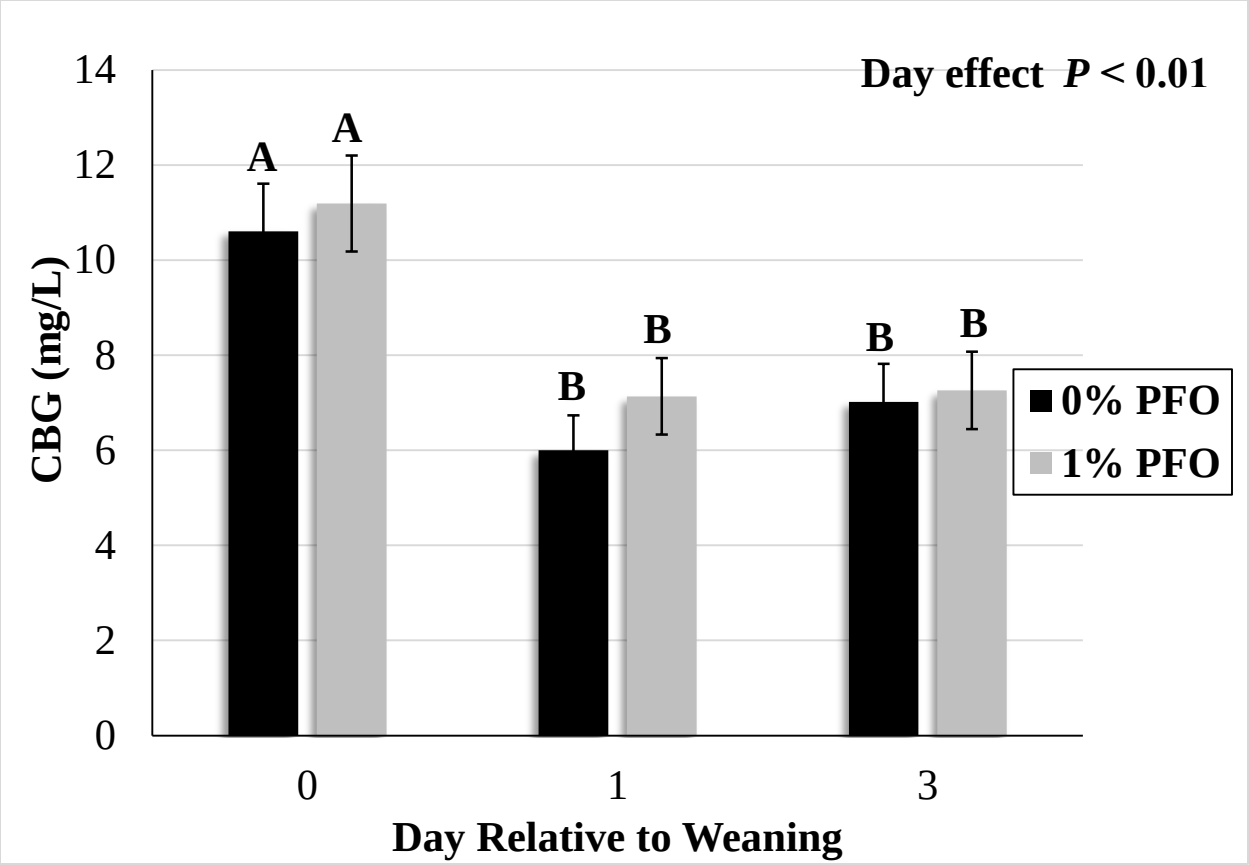


Figure 8. Plasma CBG concentrations from weaning (d 0), 1, and 3 d postweaning in pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* < 0.01) for day effect.

FCI. The FCI was lower ($P = 0.02$) for the pigs consuming the 1% PFO diet when compared to the pigs consuming the control diet over the 3 days postweaning. A treatment x time interaction was detected ($P = 0.01$) for FCI such that the FCI was lower in pigs on the 1% PFO diet versus the control on d 1. Values for pigs on either diet did not differ ($P > 0.1$) on d 0 or 3. The pigs being fed the 1% PFO diet had a greater ($P = 0.01$) FCI on d 0 than on d 1. No change in FCI was observed from d 1 to d 3. Pigs on the control diet had a greater ($P = 0.01$) FCI on d 1 when compared to d 0. Values then decreased on d 3 when compared to d 1 but were still greater ($P = 0.01$) than d 0 (Fig. 9).

Cytokines (IL-6, IL-1β, TNF-α). All three cytokines analyzed in media collected following LPS stimulation showed greater concentrations ($P < 0.01$) in collected blood than only media (Fig.10, 11, and 12). Only TNF-α tended to differ ($P = 0.098$) between treatments with the pigs on the 1% PFO diet having lower concentrations when compared to the control on d 0 (Fig 12).

WBC count, RBC count, Hematocrit, and N:L Ratio. When days were pooled the average WBC count for the control group was lower ($P = 0.05$) than the pigs on the 1% PFO diet (Fig. 13). The N:L ratio for pigs on the 1% PFO diet did not differ ($P > 0.1$) between d 0 and d 1. Pigs consuming the 1% PFO diet had a lower ($P = 0.01$) N:L ratio on d 0 than that measured for the control, with no difference ($P > 0.1$) on d 0 between the two groups (Fig. 14). Concentrations of RBCs were lower ($P = 0.01$) in pigs consuming the control diet than the 1% PFO diet on d 1 and 3. On d 0 the diets did not differ in RBC concentration. The pigs on the control diet did not differ in RBC count over the 3 days sampled. The pigs consuming the 1% PFO diet had

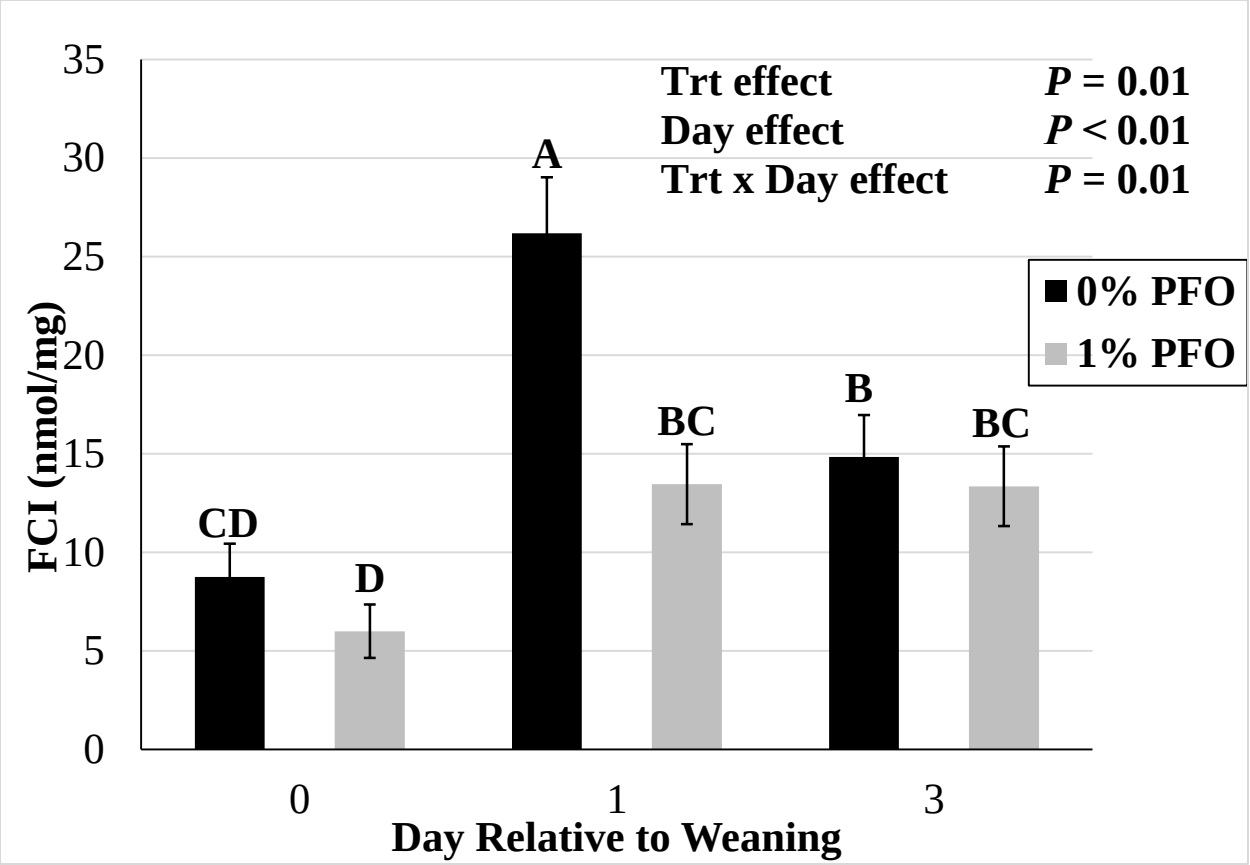


Figure 9. FCI from weaning (d 0), 1, and 3 d postweaning in pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* =0.01) representing a treatment and treatment x day effect and (*P* < 0.01) representing a day effect.

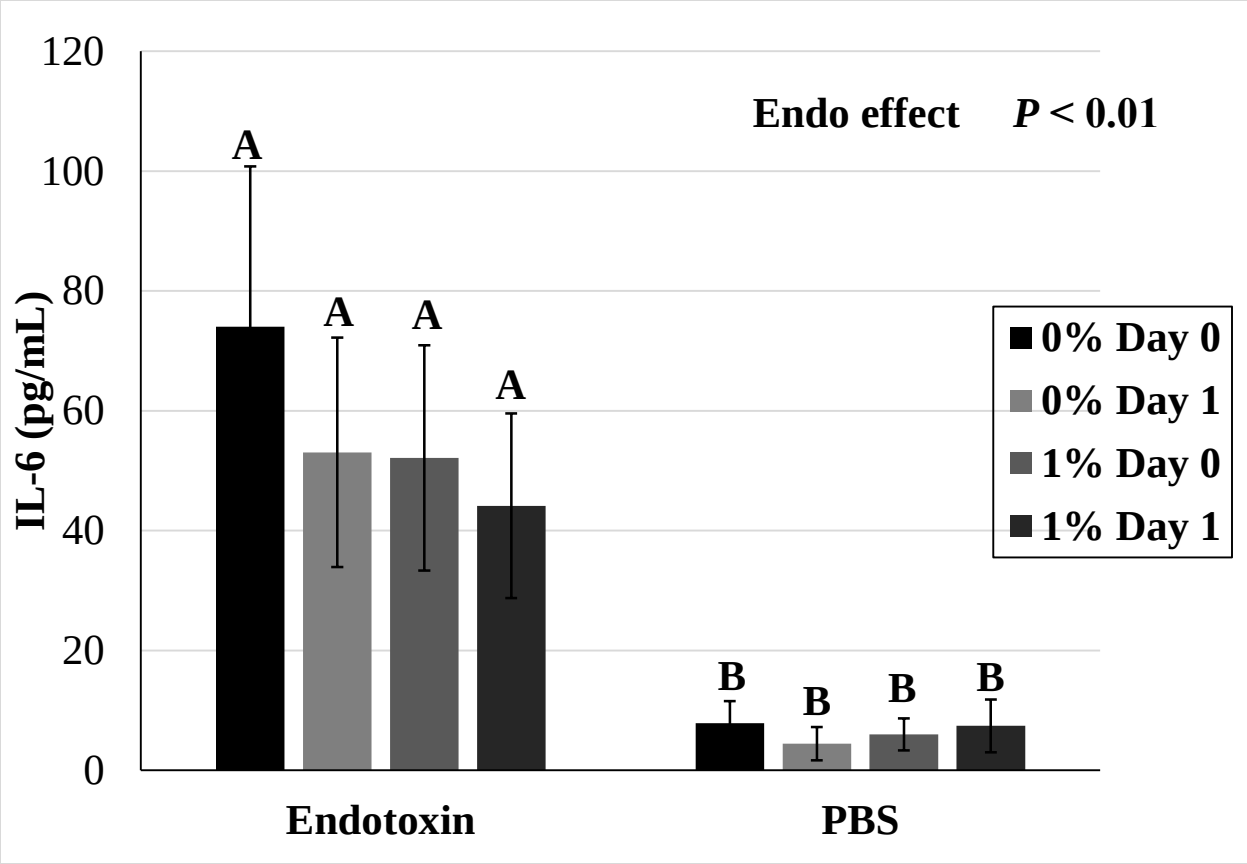


Figure 10. IL-6 concentrations when either endotoxin or PBS was added to whole blood on weaning (d 0) and 1 d postweaning from pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* < 0.01) for the endotoxin effect.

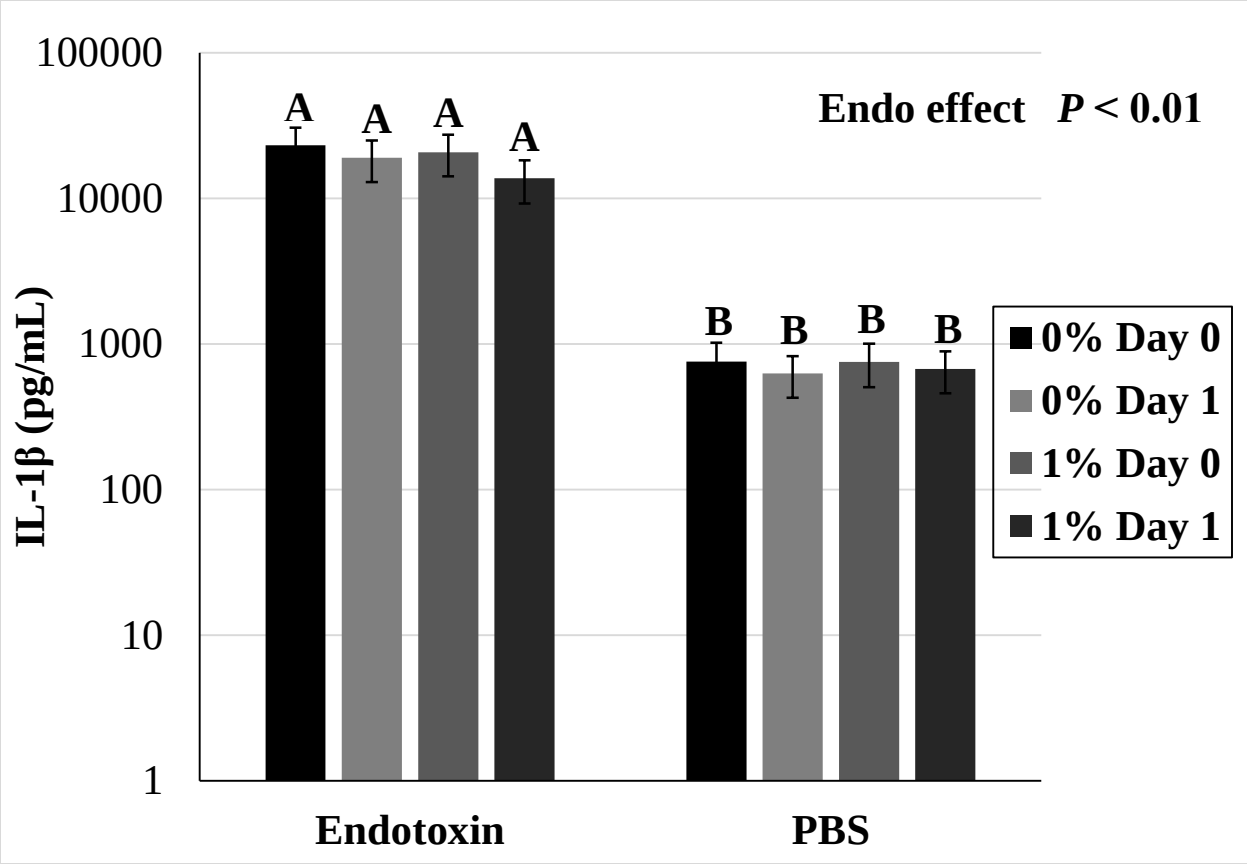


Figure 11. IL-1β concentrations when either endotoxin or PBS was added to whole blood on weaning (d 0) and 1 d postweaning from pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* < 0.01) for the endotoxin effect.

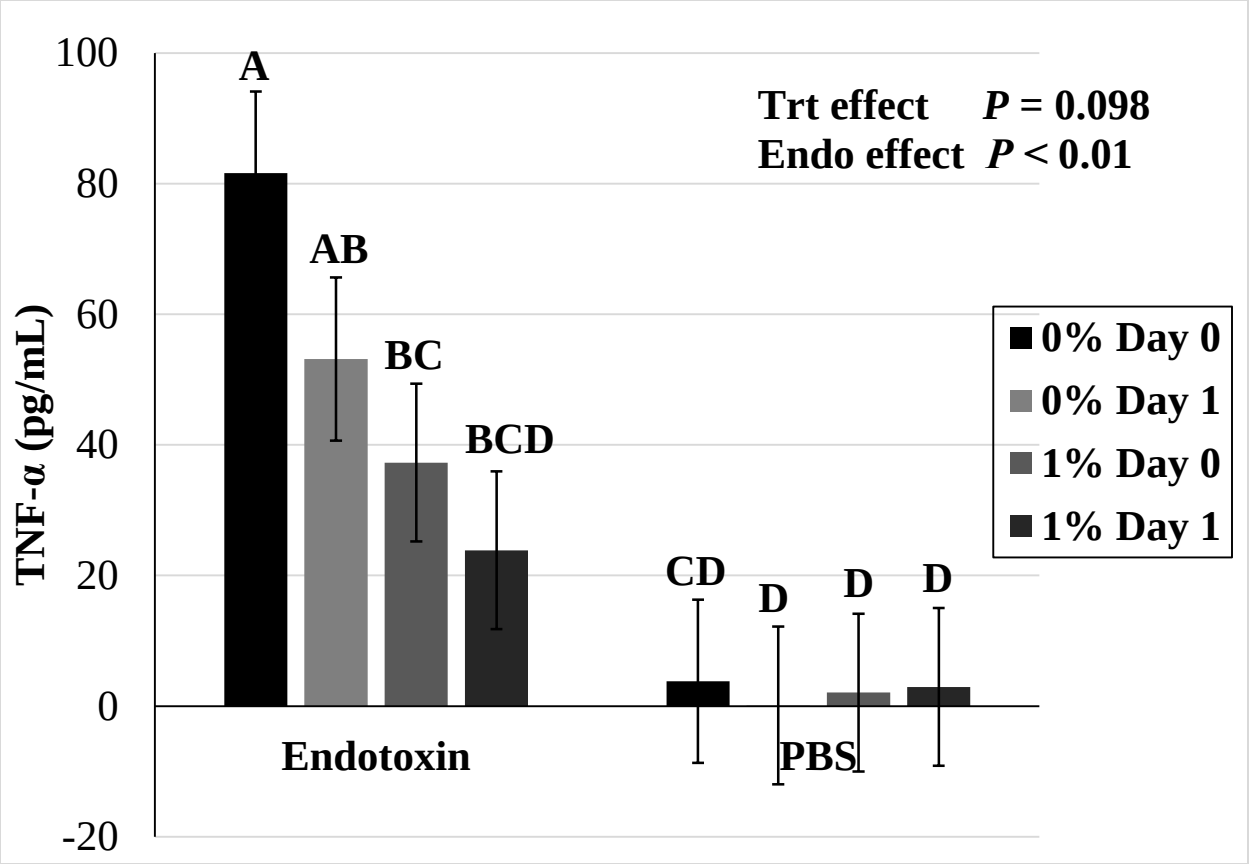


Figure 12. TNF-α concentrations when either endotoxin or PBS was added to whole blood on weaning (d 0) and 1 d postweaning from pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters tended to differ (*P* = 0.098) for the treatment effect and did differ (*P* < 0.01) for endotoxin effect.

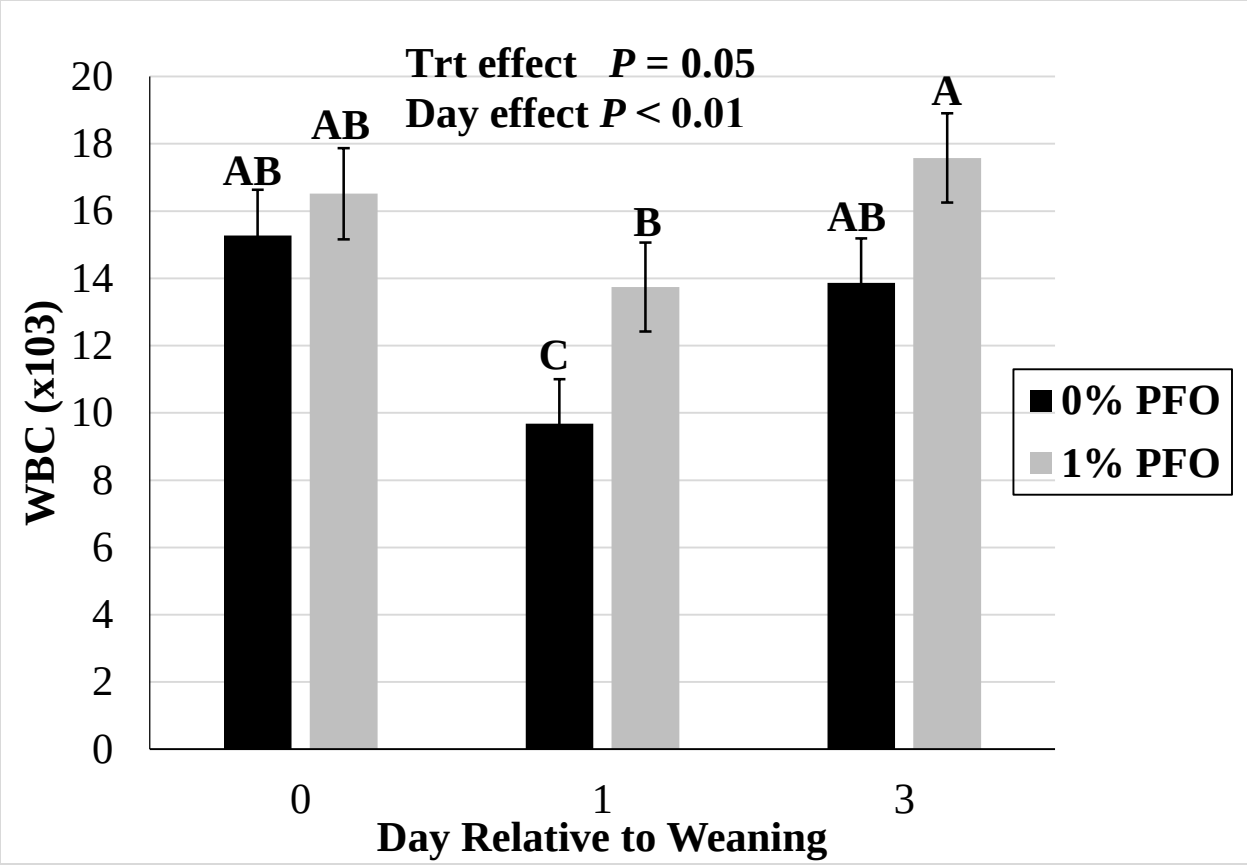


Figure 13. White blood cell (WBC) concentrations from weaning (d 0), 1, and 3 d postweaning in pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* = 0.05) for treatment effect and (*P* < 0.01) for day effect.

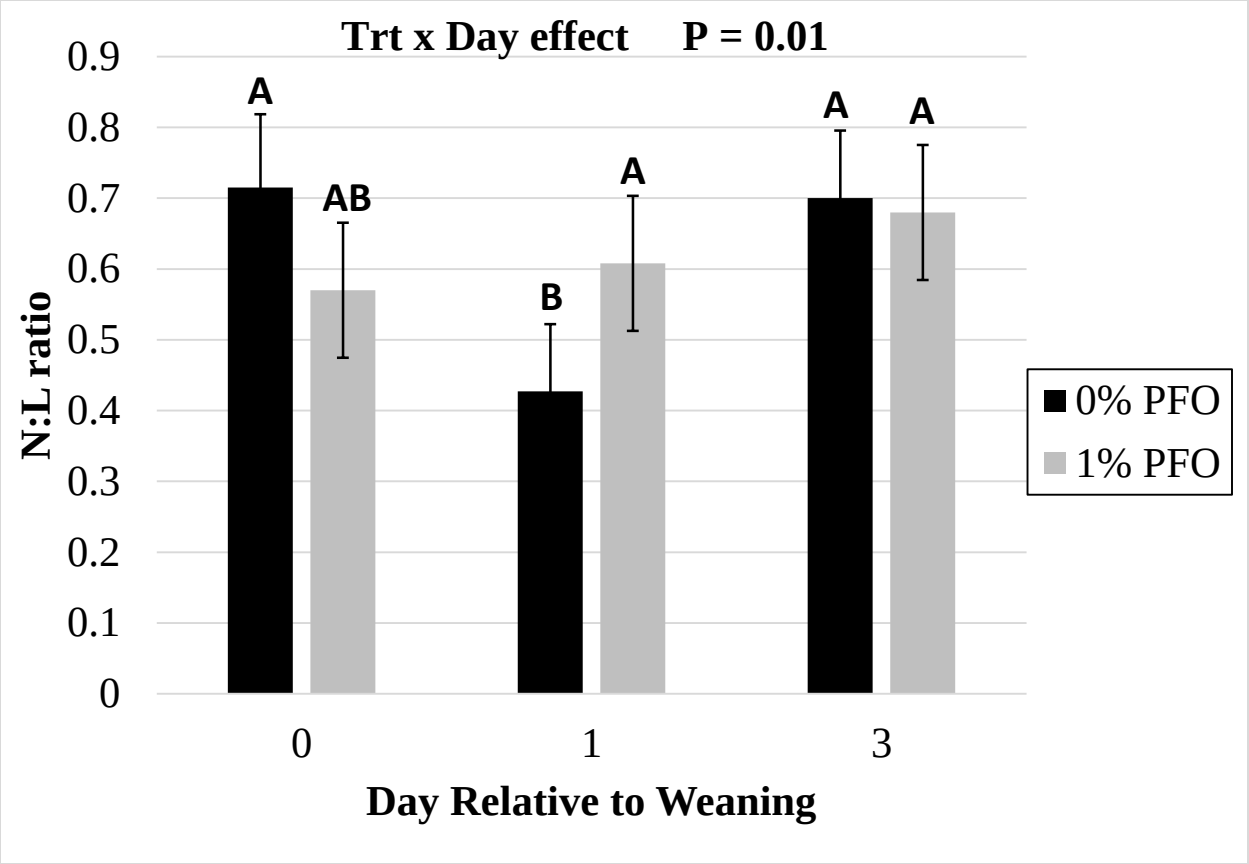


Figure 14. Neutrophil:lymphocyte (N:L) ratio from weaning (d 0), 1, and 3 d postweaning in pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* = 0.01) for treatment x day effect.

increased (P = 0.01) RBC count on d 1 relative to d 0 and did not change from d 1 to d 3 (Fig. 15). Hematocrit values for the pigs on the control diet were lower (P = 0.006) than the pigs on the 1% PFO diet over the 3 days sampled (Fig. 16).

Haptoglobin. There was an overall observed treatment effect (P = 0.02) such that haptoglobin concentrations were lower for the pigs on the 1% PFO diet than the control (Fig 17). There was also an observed tendency for a treatment x time effect (P = 0.05), such that pig haptoglobin concentrations for the control diet did not change on d 0 when compared to d 1 or d 3. Haptoglobin concentrations were lower (P = 0.05) in the pigs consuming the 1% PFO diet than the control on d 1. There was no change in haptoglobin concentration between the pigs on the 1% PFO diet throughout all three days (Fig. 17).

DISCUSSION

A multitude of fish oil varieties, from tuna oil to cod liver oil, have been used to supplement sow diets during gestation and lactation to decrease the n-6:n-3 PUFA ratio in the milk with varying results (Kim et al., 2006). Fish oil and protected fish oil, resistant to oxidation, are most commonly used for their concentrations of EPA and DHA (Horrocks and Yeo, 1999). However these n-3 PUFA are unstable due to the amount of double bonds and are subject to oxidation reducing the amount of EPA and DHA present. The auto-oxidation of these PUFA can be prevented and is done so by using fish oil protected against auto-oxidation, like Gromega™(Cameron-Smith et al., 2015). The results of the present study indicate that the addition of 1% PFO to the sow's late gestation and lactation diet reduced the n-6:n-3 PUFA ratio more than the 0.25% and 0.5% PFO diet in the sows' colostrum and milk. These results were fairly similar to those described by Gabler et al. (2007) as their 1.5% fish oil diet outperformed

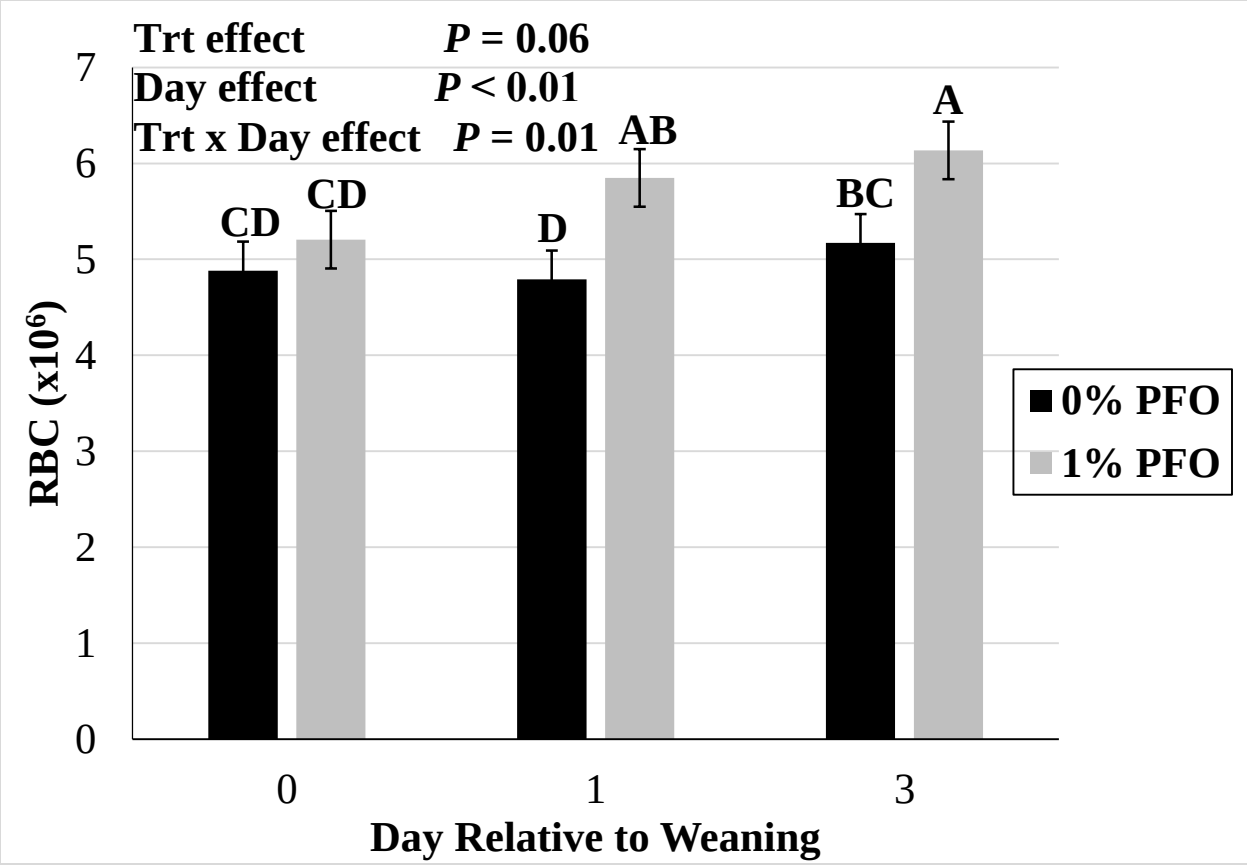


Figure 15. Red blood cell (RBC) concentrations from weaning (d 0), 1, and 3 d postweaning in pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* = 0.06) for treatment effect, (*P* < 0.01) for day effect, and (*P* = 0.01) for the treatment x day effect.

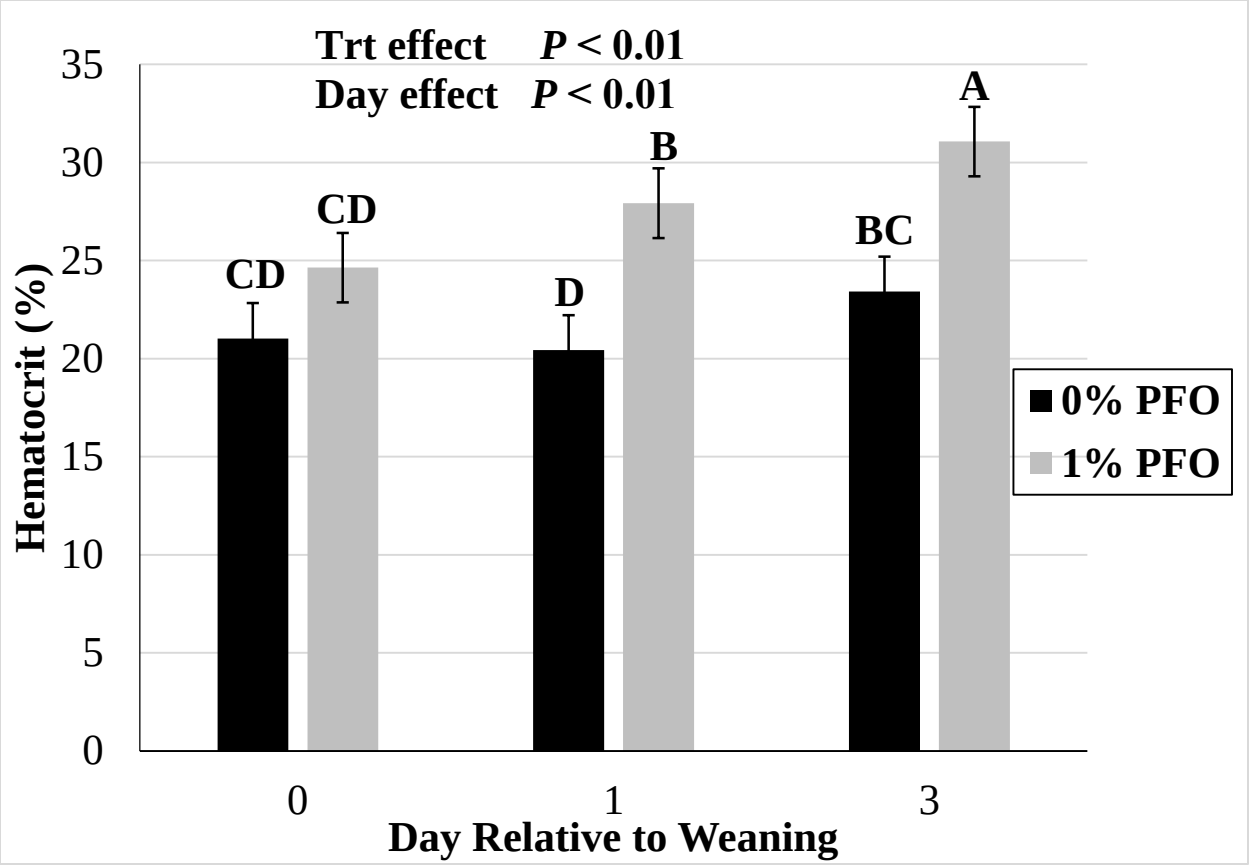


Figure 16. Hematocrit from weaning (d 0), 1, and 3 d postweaning in pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* < 0.01) for treatment and day effect.

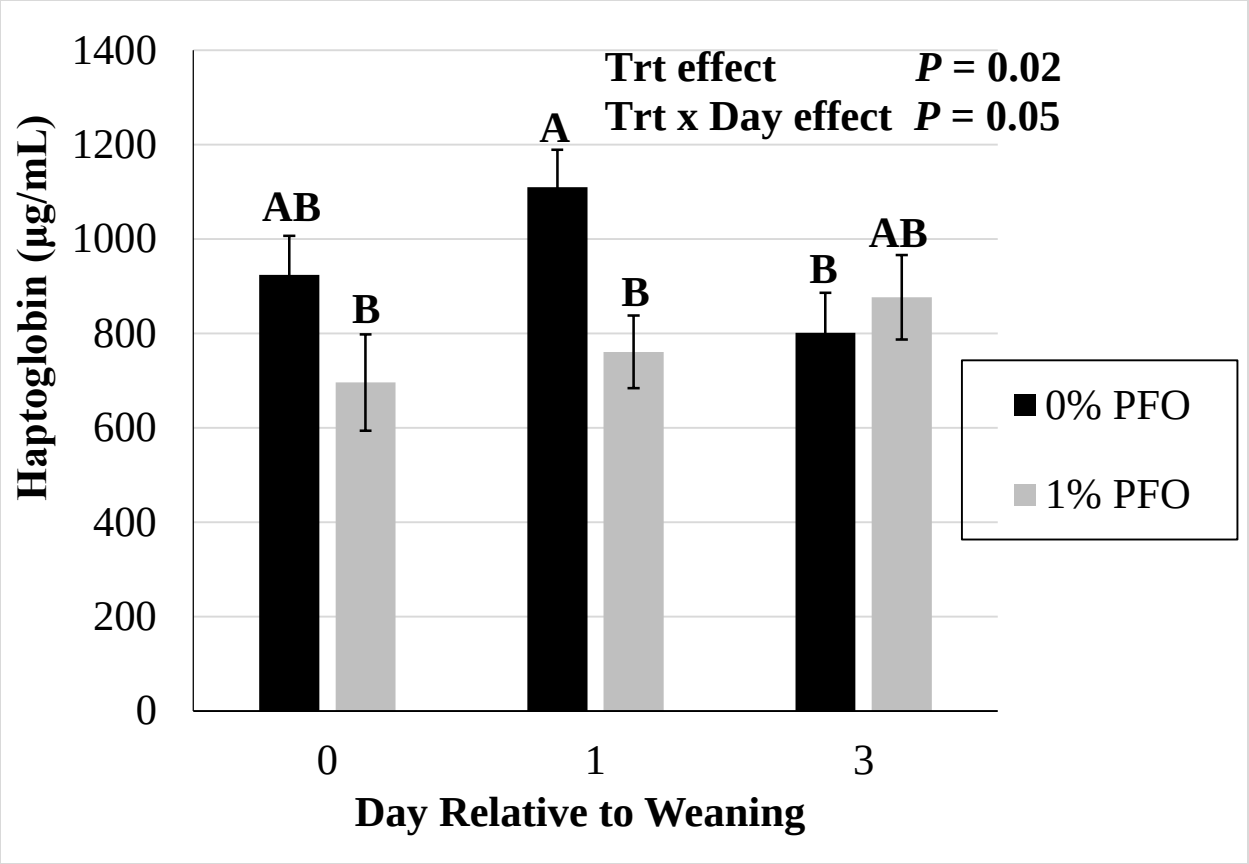


Figure 17. Haptoglobin concentrations from weaning (d 0), 1, and 3 d postweaning in pigs nursed by sows receiving a corn-soybean based diet supplemented with 1 % protected fish oil (1 % PFO, n = 16) or no supplement (0 % PFO, n = 16) 1 wk prior to farrowing until day of weaning (31 ± 2 d of age). Means ± SEM with different letters differ (*P* = 0.02) for treatment effect and tended to differ (P = 0.05) for treatment by day effect.

other similar n-3 PUFA supplements in reducing the PUFA ratio. In our study the 1% PFO supplemented diet increased the amount of DHA present in the colostrum and milk 16 d in lactation but not the EPA concentrations while the ARA concentrations remained constant in both samples. Others have observed similar results with increased DHA and EPA in milk with no effect on ARA content while supplementing with PFO (Arbuckle and Innis, 1993; Rooke et al., 1998).

In experiment 1 of the present study, the 1% PFO supplemented diet was determined to be the most effective diet in reducing the n-6:n-3 PUFA ratio of the sows colostrum and milk and, as reported by others, this reduction in n-6:n-3 PUFA ratio can be transferred to the piglet mostly through the milk (Arbuckle and Innis, 1993; Clouard et al., 2015). However, in experiment 2, the sow n-6:n-3 PUFA ratio showed no difference between the 1% PFO diet and the control. Even though sow feed consumption was not measured it was observed that two sows on the 1% PFO diet consumed notably less than the other sows on any diet. This could have affected the concentration of incorporated PUFA because the concentrations of n-3 PUFA being supplemented were 13.75% EPA and 11.39% of 39.2% total fat supplied at a rate of 5 kg/46 kg of feed. Any reduction in intake could produce undetectable differences as seen in the colostrum and milk n-6:n-3 PUFA ratio. Another cause for the lack of detectability could be due to a change in columns for the LC-MS/MS, as well as laboratory personnel unfamiliar with performing the fatty acid analysis. To test this theory, the samples will need to be analyzed using a different LC-MS/MS in another lab with more experienced personnel. It is important to note that the n-6:n-3 PUFA ratio between the 1% and control PFO supplemented diet was lower for the 1% diet in the milk samples, however this difference was not detectable statistically.

Although the sows did not show decreased n-6:n-3 PUFA ratio, in the offspring did exhibit detectable differences in plasma n-6:n-3 PUFA ratio. This would suggest that the sow's milk did incorporate enough n-3 PUFA to alter the piglets FA profile. Weaned pigs consuming the 1% PFO diet had a lower overall plasma n-6:n-3 PUFA ratio than the control pigs. The difference in n-6:n-3 PUFA ratio for piglet plasma versus sow milk could be explained by a difference in rate of tissue incorporation of total fatty acid and specific n-3 PUFA. Milk incorporation is 8 g/d of total fatty acid in the sow and pig intake of specific n-3 PUFA is estimated based upon g milk consumed and size of the pig (Gabler et al., 2007; Farmer, 2015). This could also be due to the size of the piglet in relation to the sow, as both obtain relatively the same PUFA. The increase in n-3 PUFA resulting from the effect of the 1% PFO diet, represents increased n-3 PUFA in the membrane of cells. The increase in membrane concentration increases the substrate availability for cleavage by phospholipase A2. Thus more free n-3 than n-6 PUFA is available for oxidation by COX-2, which produces a less potent series of prostaglandins, namely PGE_3, and other inflammatory mediators (Calder, 2006). The production of these less inflammatory eicosanoids and docosanoids do not as effectively stimulate the neurons leading to the HPA axis, which has been shown to result in less cortisol produced (Hong et al., 2003).

Overall the pigs consuming the 1% PFO supplemented diet weighed more after the 3 d experimental period. Not only did the pigs on the 1% PFO diet weigh more but had greater gains in body weight over the 3 day experimental period. This may be attributed to a reduction in inflammation and cortisol concentrations. Inflammation and stress due to weaning in swine decrease ADG and disrupt intestinal function, which decreases nutrient absorption (Gabler et al., 2007; Sutherland et al., 2014). This seems reasonable when considering the lower TNF-α,

haptoglobin concentrations and FCI shown presently may indicate mitigation of the inflammatory response in the 1% PFO pigs. The initial fighting after weaning causes inflammation, lethargy, and reduced feed intake (de Groot et al., 2001). A study by Giroux et al. (2000) showed that pigs with a passive reaction to stress (minimal action) had better weight gain than reacting pigs (squealing and constant movement) during the first week postweaning. This may be due to passive pigs expending less energy in their reaction to stress. However, this cannot be confirmed without evidence from video recording.

Total cortisol concentration has been shown to be a reliable predictor of a stress response but does not account for the biological activity of cortisol. As previously stated, CBG binds more than 60% of plasma cortisol in swine (Heo et al., 2005). Rising concentrations of biologically active cortisol result from increased cleavage from CBG by neutrophil elastase present at the site of inflammation (Nguyen et al., 2014). The FCI has been shown to be a dependable measure of the amount of biologically active cortisol in the circulation and a far better indices of an animals stress response (Le Roux et al., 2003; Heo et al., 2005). The present study did not show a day by treatment effect for total plasma cortisol values or CBG. Total plasma cortisol concentrations were lower for the pigs receiving the 1% PFO supplemented diet. Plasma CBG concentrations for the pigs on the 1% PFO diet were overall lower than that measured in pigs on the control diet. The lower total cortisol and greater CBG concentrations for the pigs on the 1% PFO diet and the resultant lower FCI may suggest that was pigs on this diet experienced less stress over the 3 d postweaning. The day by treatment effect for the FCI indicates that pigs regardless of treatment did experience stress on d 1 after weaning. These results are similar to those found by Kojima et al. (2008) who showed physiological responses to weaning with and without transport stress. The

control pigs in this study had increased cortisol concentration and FCI with lower CBG concentrations on d 1 after weaning. Even though both groups had higher concentrations of biologically available cortisol, the 1% PFO supplemented group had a lower FCI than did the controls. Our control pigs (0% FO diet) exhibited similar cortisol concentrations (75 vs 85 nmol/L), CBG concentrations (10 vs 6.5 mg/L), and a lower FCI (9 vs 20 nmol/mg) when compared to the control pigs (weaned and no treatment) in an experiment conducted by Kojima et al. (2008). The present study is the first to report adding 1% PFO to the sow's diet can lower FCI in pigs on d 1 postweaning.

Endotoxin challenges have been used in swine models to characterize production of acute phase cytokines IL-6, IL-1β, and TNF-α during an inflammatory response (as reviewed by Carroll et al., 2012). Direct administration of lipopolysaccharide (LPS) is a useful method to stimulate cytokine production but requires extra handling of pigs which can cause additional stress. An ex vivo whole blood LPS challenge can circumvent this additional stress. Using isolated monocytes it has been shown that upon stimulation with LPS, concentrations of cytokines produced reflect an acute inflammatory response (Boyle et al., 2006). The advantage to using whole blood is that the monocytes and other cytokine producing immune cells are in their natural environment and can more accurately represent physiological conditions within the animal whereby producing more accurate concentrations of cytokines (Damsgaard et al., 2009). In the present study, all cytokines tested showed an increase in concentration when spiked with LPS, showing that the LPS had the desired effect of inducing inflammatory conditions. However, only TNF-α differed between the control pigs and the pigs on the 1% PFO supplemented diet. Although others have reported the influence of LPS stimulation on cytokine concentrations,

these studies were completed with an *in vivo* challenge using different strains (055:B5 vs K235), timing (3 times vs 6 times) and doses (5 μg vs 2 μg) of LPS (Myers et al., 2003; Llamas Moya et al., 2006). The cytokine TNF-α may have a negative correlation with IL-1β as observed by Upadhaya et al. (2015), making it likely to be the only cytokine detectable. Also, TNF-α may be the primary cytokine activated during LPS stimulation (Upadhaya et al., 2015). In our study, pigs provided the1% PFO had lower concentration of TNF-α than did the control, overall. This suggests that the n-3 PUFA in the 1% PFO diet may have had a protective effect and reduced the TNF-α concentration during the 3 d period of acute stress. Increases in n-3 PUFA have been found to increase in the phospholipid bilayer. The effect of this increase has been shown to decrease the activity PPARγ and NFκβ. Both of which are genetic pathways that increase the concentration of inflammatory cytokines produced in immune cells (Innis, 2003; Vandoros et al., 2006). The cytokine TNF-α plays a role in the production of CBG in the liver, by upregulating CBG gene expression in hepatocytes, as well as increasing the concentration of neutrophil elastase produced from neutrophils (Emptoz-Bonneton et al., 2011; Nguyen et al., 2014). The lower concentrations of TNF-α in the pigs on the 1% PFO diet reflect the overall greater CBG concentrations observed. As a result of the higher CBG concentrations the FCI in these pigs was lower indicating less overall biologically active cortisol. Concentrations of CBG would be lower in the control due to greater TNF-α concentration decreasing CBG production in the liver. Neutrophils would also increase the production of neutrophil elastase whereby increasing concentrations of CBG with less affinity for cortisol. All of this combined would allow for higher concentrations of biologically active cortisol hence the higher FCI in control pigs.

Pigs on the control diet had higher haptoglobin concentrations overall when compared to the pigs on the 1% PFO diet. This may be due to the greater TNF-α observed in the control pigs as well as the higher concentration of biologically active cortisol. Inflammation from fighting increases the TNF-α concentrations which then increases the expression the haptoglobin in the liver (Baumann and Gauldie, 1994). Haptoglobin can be used as a marker for inflammation and has been associated with decreased growth rate in pigs (Heo et al., 2005). Our study confirms these facts, such that the control pigs had decreased gains in weight over the 3 d postweaning period with higher haptoglobin concentrations. The lower haptoglobin concentration in the pigs on 1% PFO could have been due to the lower TNF-α concentrations, which is known to increase haptoglobin production in hepatocytes (Llamas Moya et al., 2006).

There was no change in WBC count or N:L ratio from d 0 to 3 for pigs on the 1% PFO supplemented diet. The N:L ratio averaged ~0.6 which is within the normal range reported for non-stressed pigs (0.4 to 0.7; Kahn 2005) compared with that reported for pigs on d 1 postweaning (0.57). The control pigs had lower WBC counts on d 1 compared to d 0, which may have been attributed to fighting for establishment of social hierarchy and weaning stress (de Groot et al., 2001). Along with lower WBC the control pigs had a significantly greater N:L ratio on d 0 but not on day 1. The N::L ratio has been known to increase within 24 h of weaning, which has been attributed in part to elevated cortisol concentrations (Kattesh et al. 2010). This is expected because increased concentrations WBC would be associated with inflammation (Carstensen et al., 2005). As for the N:L ratio cortisol releases neutrophils in a process called demargination dramatically increasing the N:L ratio when under stressful conditions (McGlone and Pond, 2003). However, an *ex vivo* whole blood stimulation assay conducted by Carstensen et

al. (2004) observed no change in N:L ratio in pigs at weaning. The N:L ratio changes dramatically with chronic stress and pigs 24-48 h after weaning should not be experiencing chronic stress, hence the relatively level values in our pigs (Carstensen et al., 2005). When introduced to sudden and large amounts of acute stress parents of cancer patients exhibited chronic stress symptoms such as reduced glucocorticoid receptor expression, meaning the normal effects of glucocorticoids (i.e reduce production of inflammatory products) would be lessened in the presence of glucocorticoid resistant cells(Miller et al., 2002).

Normal hematocrit values for young pigs (3-10 wk) range from 25 to 35% (Kahn 2005). Pigs consuming the 1% PFO diet maintained normal values for young pigs (3-10 wk) throughout the postweaning period. The pigs on the 1% PFO diet tended to have higher RBC counts and hematocrit values than the control group across the 3 d postweaning study. A study by Bhattarai and Nielsen (2015) observed RBC count and hematocrit values at weaning and 3 wk after to have a positive association with ADG, meaning that pigs with higher RBC count and hematocrit gained more postweaning.

IMPLICATIONS

The results of the present study indicates that the inclusion of a 1% PFO supplement in the sows' diet from gestation into late lactation does have some effects on the phospholipid profile and indicators of stress and inflammation in their offspring postweaning. This study showed, albeit inconsistently, that supplementing a 1% PFO in the diet of sows does lower the n-6:n-3 PUFA ratio in colostrum and milk. This decrease although statistically undetectable can still transfer n-3 PUFA to the piglets and decrease their n-6:n-3 PUFA ratio. Through this decrease in n-6:n-3 PUFA ratio the effects of the postweaning period on acute stress and

inflammation may be mitigated. Not only did pigs fed the 1% PFO diet gain more weight but they also had lower concentrations of TNF-α, haptoglobin, and FCI all indicating a mitigation of the inflammatory and stress response associated with weaning.

Video recordings of behavior would be beneficial to view fighting bout length and frequency. Even though most of the fighting and stress happens within the first 24 h of weaning we may consider extending the postweaning period to observe if the effects of the PFO on weight gain and performance continue after the initial 3 day period.

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VITA

John M. M^{c}Afee was born into a loving family in Springfield, IL. He grew up on the Mississippi gulf coast with three other siblings. John went to Biloxi High School and upon graduation was accepted to Millsaps College. During his time at Millsaps he played football as a kicker, obtained a Bachelor of Science, and received honors in chemistry. His most valuable experience at Millsaps was the time he spent with his mentor Dr. Toyota and working on his thesis. Upon graduating he took a year off from education to help his parents and discover his next steps in life. At the end of the year John was accepted into the University of Tennessee to study and research under the direction of Dr. Hank Kattesh. During his time at UT John applied and was accepted into Purdue College of Veterinary Medicine and will be attending in the fall after graduating this summer.