## ABSTRACT

SILVA GUILLEN, YSENIA VICTORIA. Antioxidant Supplementation to Alleviate the Negative Effects of Heat and Oxidative Stress on Performance and Health of Nursery and Growing Pigs. (Under the direction of Dr. Eric van Heugten).

The present dissertation evaluated the supplementation of vitamin E, polyphenols or phytogenics in feed or drinking water on growth performance, immune and oxidative status in growing pigs housed under heat stress and in weaned piglets fed peroxidized oil diets. In study 1 (**Chapter II**) the impact of antioxidants on growth, intestinal morphology, hematology indices, immune and oxidative status in growing pigs housed under heat stress was evaluated in 2 replicated experiments. In each experiment, 64 crossbred pigs were used, with an initial body weight (BW) of 50.7±3.8 kg and 43.9± 3.6 and age of 13-weeks and 12-weeks old for each experiment, respectively. Individually housed pigs were assigned within weight blocks and sex to a 2 x 4 factorial arrangement consisting of 2 environments (thermo-neutral (21.2°C) or heat-stressed (30.9°C)) and 4 supplementation treatments (control, control+vitamin E in water, control+vitamin E in feed, and control+polyphenols in feed). Heat stress reduced growth, but no effects of supplementation were found. Intestinal morphology was not affected by heat stress or supplementation or their interaction. Intestinal cellular proliferation was increased in pigs supplemented with vitamin E in feed and water during heat stress. Serum and liver vitamin E were increased with vitamin E supplementation, especially when supplemented in water. Serum malondialdehyde (MDA), an oxidative stress marker, was greater with antioxidant supplementation compared with control, and on d 2 vs. d 28. MDA in ileal mucosa was increased when using dietary vitamin E and polyphenols. Immune status evaluated using serum cytokines was not impacted by heat stress or supplementation. Red blood cells, hemoglobin and hematocrit percentage were reduced by heat stress when measured on d 28. White blood cells, platelets, and

monocyte counts were reduced on d 28 vs. d 2. In the study 2 (**Chapter III**), the effects of dietary vitamin E and polyphenols on growth, and immune and oxidative status in weaned piglets was evaluated. Pigs (*n*=192; 21-d of age; 6.62±1.04 kg BW) were assigned within sex and weight blocks to a 2 x 3 factorial arrangement using 48 pens (4 pigs/pen). Factors consisted of lipid peroxidation (6% human-grade soybean oil or 6% peroxidized soybean oil), and antioxidant supplementation (control, control+vitamin E, and control+polyphenols). Peroxidized oils reduced growth and serum concentrations of vitamin E, especially on d 35 of the experiment. Supplementation of vitamin E increased serum vitamin E, but this increase was lower in pigs fed peroxidized oils. Serum MDA decreased with peroxidation on d 14 vs. d 35, and protein carbonyl increased by peroxidation on d 35 vs. d 14. Total antioxidant capacity (TAC) decreased with peroxidation and increased by antioxidant supplementation in the control diet. Cytokines were not impacted by peroxidation or supplementation. In study 3 (**Chapter IV**), the effects of vitamin E and phytogenics in the drinking water on growth, oxidative and immune status in weaned piglets fed peroxidized oil diets was evaluated. In a 35-day study, 21-d old weaned piglets (*n*=96; 6.10±0.64 kg BW) were assigned within sex and BW blocks to 1 of 4 dietary treatments, using 24 pens (4 pigs/pen). Diets contained either 6 % human-grade soybean oil or 6% peroxidized soybean oil. Pigs fed peroxidized lipids received drinking water without (control) or with supplemental vitamin E or phytogenics. Peroxidized oil diets reduced growth. Serum vitamin E increased with vitamin E supplementation compared with other treatments. 8-hydroxydeoxyguanosine and protein carbonyl (oxidative stress markers) decreased and increased, respectively, by phytogenic treatment and control peroxidized treatment. MDA and TAC increased on d 35 vs. d 7. Cytokines were not impacted by day of collection or treatments. Supplementation of antioxidants in feed or

drinking water did not alleviate the negative effects of heat and oxidative stress on growth performance and health on growing pigs and weaned piglets.

# Antioxidant Supplementation to Alleviate the Negative Effects of Heat and Oxidative Stress on Performance and Health of Nursery and Growing Pigs

by
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## DEDICATION

I dedicate this PhD dissertation to my mom Emiliana Guillen, my dad Victor Silva, my sister Emily, my brothers Ivan and Kevin, and my nephew Matheus who were the principal emotional support of this new academic achievement. Thanks, beloved family for your support and advice!

Dedico esta tesis de doctorado a mi mamá Emiliana Guillen, a mi papá Víctor Silva, a mi hermana Emily, a mis hermanos Iván y Kevin, y a mi sobrino Matheus quienes fueron mi principal apoyo emocional en este nuevo logro académico. ¡Gracias, querida familia por su apoyo y sus consejos!

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## BIOGRAPHY

Ysenia Victoria Silva Guillen was born on March 6th, 1985 in Lima, Perú. Her father is Victor Silva and her mother is Emiliana Guillen. In Aug 2004, she was admitted to the National Agrarian University La Molina (UNALM) in Lima. During her undergraduate studies, she did various internships in different pig farms where she gained experience in pig production. She received her B.Sc. degree in Animal Science in 2009 from UNALM. In 2010, she worked on a pig farm as a manager in Lima. In Jul 2011, she received her Engineer degree in Animal Science by the same University after defending her thesis in swine nutrition under the direction of Eng. Carmen Alvarez. She started her Masters studies in March 2012 at State University of Sao Paulo (UNESP) in Jaboticabal, Sao Paulo, Brazil, funded by a Master Scholarship from the Brazilian Government. Her research focused on swine nutrition under the direction of Dr. Maria Cristina Thomaz. Ysenia received her M.Sc. degree in Animal Science in May 2014. In Aug 2014, she started to work as Instructor at Scientific of the South University and an Agricultural College in Lima dictating lectures on pig production and animal nutrition & feeding, respectively, and she did consultant activities for small Peruvian pig producers. Finally, she started her Ph.D. studies in Aug 2016 at North Carolina State University, Department of Animal Science, under the direction of Dr. Eric van Heugten, funded by a PhD scholarship from the Peruvian Government. Her research focused on antioxidants in pigs exposed to heat and oxidative stress.

"Working with pigs is not a job, it is her passion".

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# CHAPTER I: LITERATURE REVIEW

## INTRODUCTION

The world is facing a high ambient temperature phenomenon denominated global warming. Global warming causes stress in livestock, which happens when pigs are exposed to temperatures that are above their thermal comfort zone. Upon high temperature exposure (over $25^0$C), pigs will attempt to decrease their body heat production and increase body heat loss to maintain core temperature. Panting, increased respiratory frequency, increased rectal temperature and reduced feed intake are mechanisms used by pigs to dissipate heat stress (Campos *et al*., 2017). Heat stress in growing pigs compromises voluntary feed intake, performance and health, including intestinal integrity. In the US, heat stress causes economic losses in growing-finishing pigs due to decreased performance and increased mortality (St-Pierre *et al*., 2003). In North Carolina, one of the largest pig producing states, it represents 2.9 kg of lost gain per pig per year, and in the US, it represents losses over $300 million per year (St-Pierre *et al*., 2003). Heat stress redistributes blood flow to the periphery to release excess heat, causing intestinal damage, reduced absorptive capacity, and increased permeability, allowing pathogens to enter the bloodstream. Heat stress produces an imbalance in the natural antioxidant system and oxidants in the body provoking oxidative stress. Similarly, oxidative stress can be induced by feeding of peroxidized lipids associated with poor lipid quality, which has a negative impact on redox balance and membranes of enterocytes.

In 2017, the production of oil in the United States was 571 million metric tons, and from 1998 to 2017 has been increasing around 15% annually (Statista, 2019). Oils provide a high concentration of energy in animal diets. Consumption of vegetable oils worldwide is 197.3 million metric tons as estimated for the 2018/2019 period. Soybean oil is one of the most common oil types consumed by humans for cooking (Statista, 2019). Cooking and heating produces lipid

peroxidation. This process occurs when an unsaturated fatty acid is attacked by oxygen molecules forming free radicals or reactive oxygen species (ROS) causing oxidative stress to lipids of cell membranes (Buettner, 1993; Girotti, 1998; Kerr *et al*., 2015).

Oxidative stress is defined as a high concentration of reactive oxygen species (ROS) within cells causing alterations to their normal functioning if they cannot be balanced with antioxidants. Antioxidants quench oxidative processes by donating electrons to oxidants, and the oxidized antioxidants are recycled (Lykkesfeldt and Svendsen, 2007). Moreover, dietary antioxidants like vitamin E and dietary polyphenols have shown beneficial effects against heat and oxidative stress in humans, pigs and poultry (Brenes *et al*., 2008; Pandey and Rizvi, 2009; Lu *et al*., 2014ab; Akbarian *et al*., 2016; Liu *et al*., 2016; Zhang and Tsao, 2016; Ebrahimzadeh *et al*., 2018). This review will focus on the mechanisms involved during heat stress and lipid peroxidation and their effects on health and pig performance, and the potential role of vitamin E and polyphenols as antioxidants to alleviate negative impacts of heat and oxidative stress.

## HEAT STRESS

### *Economic impact of heat stress in swine*

Global temperature is increasing each year, according to the Intergovernmental Panel on Climate Change (IPCC, 2019). Temperatures are rising from 1990 to 2100 in the order of 1.4 to 5.8°C (Mendelsohn and Williams, 2004). Under this condition, the environment and animal production will be increasingly impacted due to high temperatures (IPCC, 2019). Reductions in body weight gain and increased time required to reach market weight can be considered the principal factors impacting the animal industry. In the US, reductions in growth performance, mortality and economic losses annually in growing-finishing pigs represent around 2.4 kg per pig, 1.3 pigs per 1000 pigs, and $202 million, respectively. North Carolina is one of the largest pig

producing states and the most affected state in the US due to heat stress. Growth loss, mortality, and economic losses are around 2.9 kg per pig per year, 1.6 per 1000 pigs, and $42.9 million per year, respectively (St-Pierre *et al*., 2003). All these facts suggest that the reduction in the production of animal protein by heat stress will impact the availability of meat for human consumption.

***Physiology of heat stress in pigs***

A normal diet provides energy and nutrients for maintenance, locomotion, and growth under thermo-neutral conditions. However, below the critical temperature (LCT; defined by Mount (1975) as the lowest environmental temperature at which a pig can maintain thermoregulation at a minimal metabolic rate) the metabolic heat increases to meet normal core temperature, i.e., augment energy consumption and generating an equilibrium between energy balance and growth. Contrarily, pigs housed above the critical temperature experience a rise in body temperature and metabolic rate, producing thermoregulatory failure (Mount, 1975), reducing energy intake, energy retention and locomotion, and increase heat loss (Quiniou *et al*., 2001). In the 70's, the LCT was defined at 18ºC, but modern pigs (60 kg of BW) are much leaner than pigs from the 70's, and are more sensitive to cold and heat. Henceforth, increased energy expenditure starts when the environmental temperature is lower than 23 to 24 ºC, which is the LCT for modern growing pigs between 30 and 90 kg, and it requires 19 g/d per ºC from 12 to 24 ºC environmental temperature (Quiniou *et al*., 2001).

The term "thermal comfort" refers to the relationship between an individual and its response to the environment. The thermal comfort zone is the range of environmental temperatures when metabolic rate is constant and the body does not need to expend energy to maintain thermal homeostasis because heat produced is in equilibrium with heat loss (Joshi *et al*., 2016).

The comfort temperature in pigs depends on BW (light or heavy), age (piglets vs. finishing pigs), and housing (individual vs. group) (Renaudeau *et al*., 2011). Thus, an individually housed pig, during low temperature, needs to produce additional heat in comparison with group housed pigs to maintain normal body temperature (Quiniou *et al*., 2001). High environmental temperatures affect ADFI, ADG and feed:gain ratio in pigs. For example, 10-kg and 90-kg pigs started to have decreased ADFI at 30 and 21ºC, respectively. ADG was reduced starting at 29.3ºC in 10-kg pigs and from 22.9 ºC in 90-kg pig. The feed:gain ratio in pigs is associated with the reduction in ADFI due to high temperature effects (Renaudeau *et al*., 2011).

Heat transfer coefficients are used to estimate heat exchange, and they depend on temperature differences and include radiation, convection, and conduction. Radiant heat exchange is the surface temperature corresponding to black body temperature (in which a body reflects electromagnetic radiation). Convective heat exchange occurs when wind or air hit the surface of the body and the warmed air rises from the skin due to its low density. Additionally, conductive heat exchange occurs when a pig lying on slats or ground inducing heat loss per unit area by radiation and convection (Mount, 1978).

Heat stress is the result of the net amount of corporal energy interacting with the environment and the amount of heat produced by pigs causing a negative heat balance. Pigs with high genetic potential for lean growth experience high heat stress levels due to their higher maintenance requirements associated with increased muscle. Negative implications were also observed in sows, e.g., decrease in feed intake, milk production, litter body weight gain and fertility and in gilts at breeding having decreased fertility (St-Pierre *et al*., 2003).

***Heat stress and effects on voluntary feed intake***

Heat stress reduces voluntary feed intake (Quiniou *et al*., 2000, Baumgard and Rhoads, 2013, Pearce *et al*., 2013a and Campos *et al*., 2017), average daily gain and gain:feed ratio in growing pigs (Kerr *et al*., 2003) and this effect was associated with an alteration in skeletal muscle physiology (Montilla *et al*., 2014), causing a reduction in muscle deposition and increased adipose tissue gain (Collin *et al*., 2001).

The reduction of feed intake is associated with a reduction in heat production under high environmental temperature. Thus, pigs of 60 kg and 80 kg BW decreased feed intake by 50 and 80 g/d, respectively, when housed at 24 to 30ºC (Campos *et al*., 2017). According to Pearce *et al*. (2013a), heat stressed pigs reduced their feed consumption by 47% when compared with thermo-neutral pigs. This reduction is due to a strategy of the pig to minimize metabolic heat production associated with feed metabolism. Furthermore, the reduction of total heat production is caused by increased energetic efficiency due to a reduction in fasting heat production in growing pigs housed in a heat stressed environment (Collin *et al*., 2001).

The reduction in feed consumption reduced growth in heat-stressed pigs and reduced circulating glucose, but increased circulating insulin concentration. This increase in insulin concentration stimulates accumulation of lipids in the carcass of heat stressed pigs. In addition, the accumulation of lipids is associated with a reduction in metabolic heat production because energetic efficiency of lipid deposition is greater than protein deposition (Campos *et al*., 2017 and Baumgard and Rhoads, 2013). Furthermore, fatty acid utilization is reduced and aerobic glycolysis to generate ATP is increased in heat stressed pigs (Pearce *et al*., 2013a).

Internal organ weight of the heart, pancreas, stomach and large intestine were reduced in pigs housed under heat stressed conditions, which is consistent with the reduction in feed

consumption and growth rate (Kerr *et al*., 2003 and Johnson *et al*., 2015b). Similarly, under heat stress, a reduction in liver weight (1.33%), spleen (0.17%), and blood (4.2%) with respect to empty BW (final live BW without gastrointestinal contents) was reported by Johnson *et al*. (2015a).

The thyroid gland regulates growth performance and adaptations in the body. It stimulates lipogenesis and lipolysis, influencing metabolic pathways that control energy balance (Mullur *et al*., 2014). T4 (thyroxin) is a prohormone and serves as primary secretory product of the thyroid gland. T4 is converted to T3 (triiodothyronine) providing negative feedback to endocrine cells in the anterior pituitary to regulate thyroid hormone (Mullur *et al*., 2014).

As T3 is considered thermogenic and both T3 and T4 stimulate phosphorylation and ATP transport, a decrease of the thyroid hormone concentrations (T3 and T4) is related to a decrease in metabolic rate and a reduction in metabolic heat production (Collin *et al*., 2002). Under high temperatures, a reduction in T3 and T4 concentration occurs, decreasing the metabolic rate and heat produced by cells (Campos *et al*., 2014). In addition, endogenous T3 and T4 concentrations were lower in growing pigs exposed to high temperatures (32ºC) (Macari *et al*., 1986; Campos *et al*., 2014).

***Heat stress and effect on rectal temperature and respiration rate***

Respiration is the interchange of oxygen and $CO_2$ between external environment and the body through the lungs. Additionally, during this process, evaporative heat transfer, which is the difference of vapor pressure between the environment and the skin surface, occurs (Mount, 1978). Measurement of respiration rate was described by Quiniou and Noblet (1999) as counting flank movements over a one-minute period by an observer avoiding any physical contact with the pig. In a study by Pearce *et al*. (2013a), respiration rate was elevated in heat stressed pigs when compared to thermo-neutral housed pigs (115 vs. 55 breaths per minute).

In an experiment reported by Renaudeau *et al*. (2010), respiration rate of pigs under different environmental temperatures (24, 28, 32 and 36 °C) were evaluated during 20 days. Results showed increased respiration rate in pigs housed at 36 °C vs 24 °C during the first 2 days (acute heat stress). From day 2 to 4 a reduction in respiration rate at 28, 32 and 36 °C was observed. Pigs under chronic heat stress after day 4 maintained the same rate until day 20. These results clearly indicate that heat stress during acute or chronic heat stress was not linear.

The normal rectal temperature in growing and finishing pigs is 39.2°C, ranging from 38 to 40°C, depending on the individual pig and conditions (Campos *et al*., 2017). Heat exposure increased rectal temperature in growing pigs from 39.3 to 40.8°C in a study conducted by Pearce *et al*. (2013a). Johnson *et al*. (2015a) showed that growing pigs, from 30 to 60 kg BW, exposed to constant high temperatures (34°C) had high respiration rate compared with control pigs (94 vs. 49 breaths per minute) and increased rectal temperature (39.4 vs. 39.0 °C), respectively. The same authors (Johnson *et al*., 2015b) in another paper, reported increased rectal temperature and respiration rate compared with control (39.6 vs. 39.3 °C and 92 vs. 58 breaths per minute), respectively, in finishing pigs (from 62 kg BW to 80kg BW). Renaudeau *et al*. (2010) reported that acute and chronic high temperature exposure resulted in two types of responses in growing pigs. During acute exposure for 1 day from 24 to 32 °C, a high respiration rate and rectal temperature was observed, with a decline the following 2 days and a constant respiration rate up to the end of the period.

***Heat stress and intestinal health***

The principal function of the gastrointestinal (GI) barrier is to regulate the absorption of nutrients, water, and electrolytes from the intestinal lumen into the bloodstream (Kelly *et al*., 2015). At the same time, the GI barrier protects the internal body environment from bacteria and

lipopolysaccharides (LPS), which are components of the cell wall of gram-negative bacteria (endotoxin).

Hyperthermia induced by heat stress affects the GI barrier causing increased intestinal permeability due to a redistribution of blood flow from the GI tract to the periphery to dissipate heat to the environment. GI ischemia is due to a drastic reduction of blood flow, increasing vascular permeability (Leon, 2007). Also, the GI tract is affected by lower amounts of oxygen (hypoxia) at the intestinal epithelium, compromising its function and integrity (Sanz Fernandez *et al*., 2015), and causing inflammation, affecting tight junction proteins such as occludin and claudin. Hypoxia also facilitates the leakage of bacteria, releasing endotoxins, into the bloodstream causing endotoxemia, cytokine release, and free radical production, ultimately causing barrier disruption and affecting intestinal villi (Leon, 2007) as shown in Figure 1.

The intestinal epithelium is protected by tight junctions (TJ), adherens junctions (AJ), and desmosomes. The function of TJ is restricting entry and normal transport of molecules and maintain cell polarity. Also, TJ function as transcellular and paracellular barrier restricting entry of bacteria and endotoxins (Dokladny *et al*., 2006). TJ proteins are formed by occludins (limiting the stability of micelles and microcellular flux), claudins (form paracellular channel) and junctional adhesion molecules (JAM) (Bhat *et al*., 2018). A proposed model by Lingaraju *et al*. (2015) suggests that lipids are part of their structure and influence the functionality of tight junctions.

When the TJ barrier is compromised, intestinal permeability is increased causing erosion and ulceration. This causes an influx of immune cells into the mucosa, stimulating the release of pro-inflammatory cytokines in the mucosa.

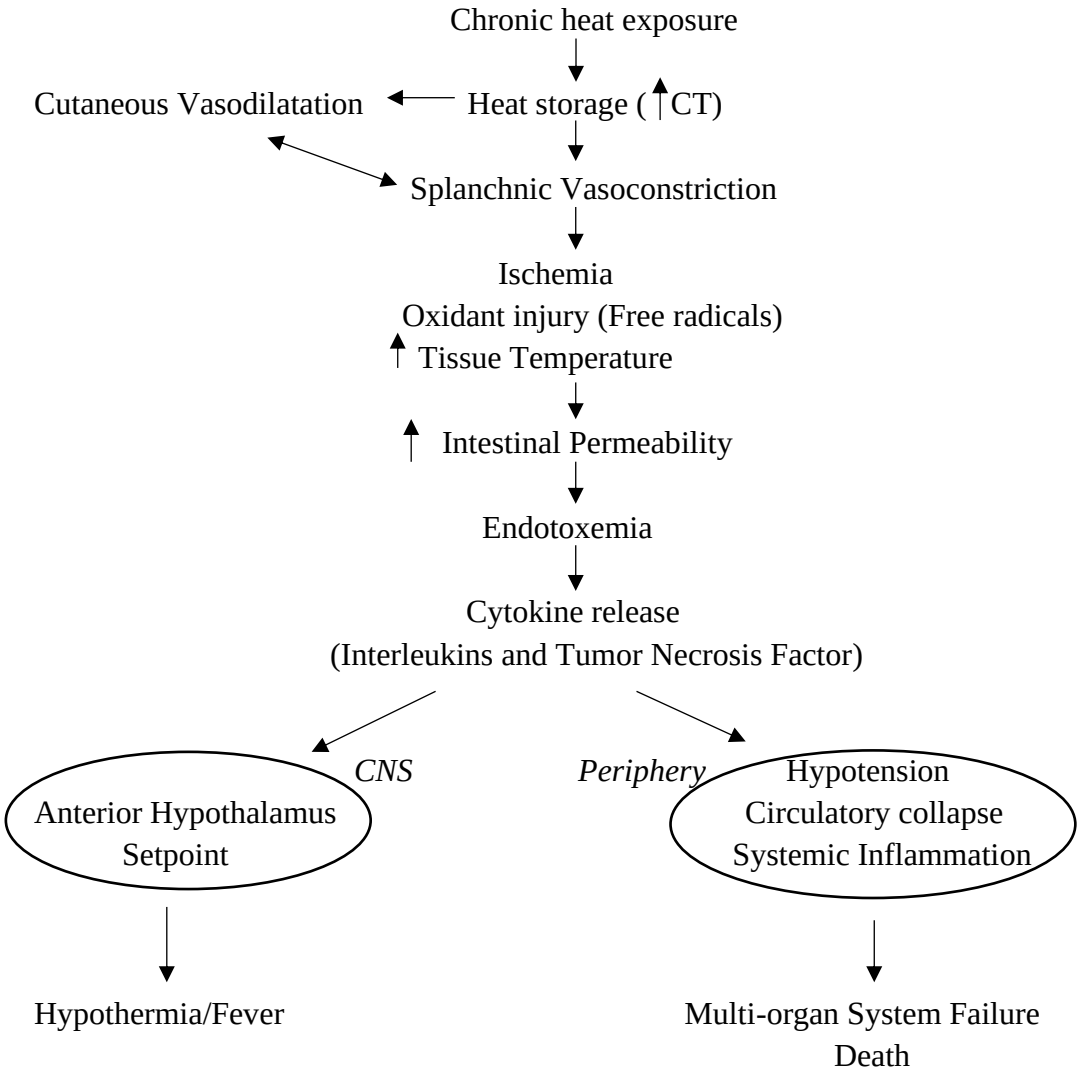


**Figure 1.** Effect of chronic heat exposure on blood flow, intestinal permeability and cytokines. Adapted from (Leon, 2007). Environmental chronic heat stimulates increases in core temperature (CT), increasing cutaneous blood flow, facilitating heat dissipation. Cutaneous vasodilation is the consequence of splanchnic vasoconstriction representing a compensatory response. Ischemia of the intestine causes oxidative stress in tissues, leading to free radical production, causing intestinal permeability. Henceforth, membrane permeability leads to endotoxin leakage and cytokine stimulation (IL-1, IL-6, and IL-10). Cytokine production signals stimulate to the Central Nervous System (CNS), which induces changes to core temperatures leading to fever and hyperthermia. In addition, cytokines that are produced stimulate inflammation and multi-organ failure and death.

AJ are located between cells and is a form of cell to cell anchor. The principal function of AJ is maintaining physical contact between cells avoiding disruption and tissue disorganization in cells maintaining intercellular homeostasis (Meng and Takeichi, 2009). Desmosomes provide

strength to the cell creating a strong bond between the cell and the cytoskeleton. Additionally, gap junctions are channels permitting the passage of nutrients (Bhat *et al*., 2018). Epithelial intercellular junctions in mammals are shown in Figure 2.

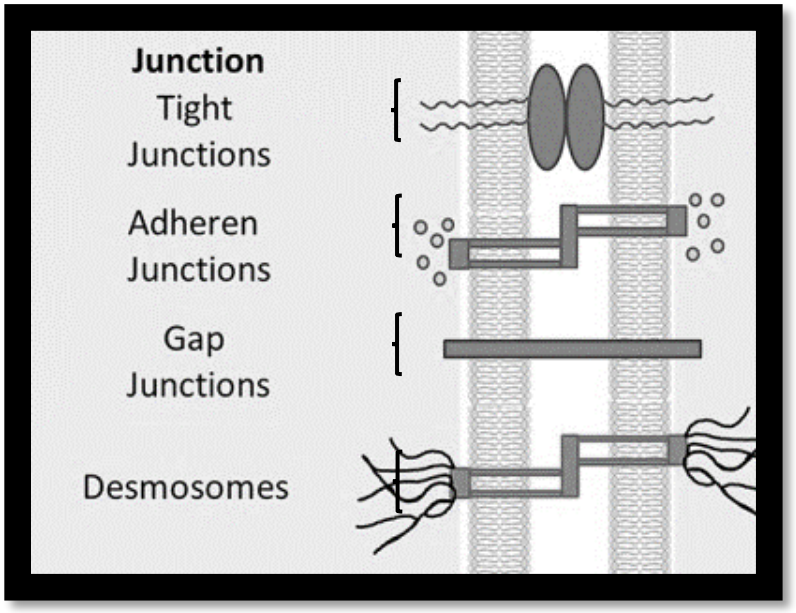


**Figure 2.** Epithelial intercellular junctions. Adapted from Bhat *et al*. (2018). Distribution of epithelial intercellular junctions from the apical region to the basal region.

Acute heat stress induces apoptosis of cells. Apoptosis is the normal cellular death in the body. Apoptosis has been observed in the thymus, spleen, lymph nodes, and small intestinal mucosa during heat stress stimulation (Gaffin and Hubbard, 1996). Lipopolysaccharides (LPS) are components of the cell wall of gram-negative bacteria that induce apoptosis in intestinal cells.

Apoptosis increases intestinal permeability, allowing bacteria to enter into the bloodstream causing systematic inflammation and injury to organs (Liu *et al*., 2017). High concentrations of endotoxins in blood can be found when the GI barrier was disrupted by heat stress (Leon, 2007). High LPS concentrations in blood were found during intestinal permeability by heat stress exposure (Sanz Fernandez *et al*., 2015). LPS has the potential to trigger the inflammatory process

in heat stressed pigs by activation of the NF-κB pathway (an inflammatory signaling pathway) (Montilla *et al*., 2014).

***Heat stress and immune status (cytokines)***

Cytokines are protein cell regulators that control immune responses in the body and are released by macrophages, T and B cells and endothelial cells (Leon, 2007). Cytokines are divided into two types, pro- and anti-inflammatory cytokines. Interferon (IFN)-γ, tumor necrosis factor (TNF)- α, interleukin (IL)-1β, IL-2, IL-6, and IL-8, IL-12 are pro-inflammatory cytokines and IL-4 and IL-10 are anti-inflammatory cytokines (Koorts *et al*., 2011). Cytokines interact with specific high-affinity receptors but can be toxic when they are present in high amounts (Gaffin and Hubbard, 1996). Ji *et al*. (2018) showed the presence of an intestinal α-defensin called defensin-related cryptidin-2 (Cry-2), produced by Paneth cells. Paneth cells have the innate role to protect the mucosa against pathogens. Cry-2 is upregulated during heat stress and proportionally increased in response to stress in mice. In addition, ulinastatin (multivalent enzyme inhibitor) protects the intestine from heat stress-induced intestinal damage, by regulating Cry-2 expression and decreasing TNF-α.

IL-10 is an anti-inflammatory cytokine with a potent inhibitory capacity on the production of pro-inflammatory cytokines such as IFN-γ, IL-1, IL-6, and TNF. IL-10 and IL-6 both are produced by Th2 (T helper 2) cells, monocytes, B cells, and muscle tissue for IL-6. (Koorts *et al*., 2011). High IL-6 concentration was correlated with high intestinal permeability in mice (Wang *et al*., 1996). IFN-γ is a signaling protein produced by host cells in response of pathogens (Taïwe and Kuete, 2017). IFN- γ, under endothelial barrier disfunction due to heat stroke in humans, increased molecular cell adhesions which are composed by a group of proteins located on the cell surface helping to stick cells together during the adhesion process (Taïwe and Kuete, 2017).

Increased plasma IL-1β concentrations in the hypothalamus and plasma in rabbits were associated to high temperature exposure (42.8ºC) (Lin *et al*., 1994). IL-1β is produced by innate immune system cells; the secretion is due to inflammatory stimulus (Leon, 2007). IL-1β, as pro-inflammatory cytokine, is produced by intestinal cells (Kruse *et al*., 2008). IL-1β, IL-6, IL-10 and TNF are produced upon hypothalamic-pituitary-adrenal (HPA) axis activation, vascular permeability, shock and death (Leon, 2007).

Heat shock proteins (HSP's) are proteins that acts as molecular chaperones facilitating protein folding for denatured proteins and prevent irreversible denaturation (Dokladny *et al*., 2006). They also modulate the production of cytokines during stress.

HSP are between 27,000 to 110,000 Da in size and belong to a huge family of 60 different proteins. For example, HSP 70 has a protective function during thermal stress, ischemia and tissue injury, facilitating folding of newly synthetized proteins (Dokladny *et al*., 2006 and Leon, 2007).

In a study by Pearce *et al*. (2013a), the level of HSP 70 was increased in a heat stressed environment when compared with thermo-neutral pigs. During elevated external and core temperatures, the body synthesizes HSP and interacts with cytokines to regulate heat stress. Exposure to high heat stimulates the release of HSP, which was shown to protect cells during the inflammation process in rats (Heidemann and Glibetic, 2005).

TNF-α is considered as a pro-inflammatory cytokine and induces fever and anorexia (Leon, 2007). The principal cytokine during heat stress is TNF-α. TNF-α, as the biologically active form, is produced by macrophages and monocytes in the presence of LPS (Kruse *et al*., 2008. Contradictorily as aforementioned, serum TNF-α was decreased in growing pigs during acute heat stress (24 h of high temperature exposure at 35ºC) and diurnal heat stress (6h at 38 ºC and 18h at 32ºC) (Pearce *et al*., 2013b and Gabler *et al*. 2018).

IL-1 is a pro-inflammatory cytokine produced by macrophages, monocytes, and fibroblasts. During inflammation, IL-1 is released into the local environment (Taïwe and Kuete, 2017). IL-6 showed elevated concentrations during heat stress, reduced gastrointestinal permeability and has protective properties under high temperature exposure, preventing damage and inflammation in the intestine of mice (Phillips *et al*., 2015).

***Heat stress and hypothalamus activation***

Swine are a homeothermic species that can maintain core temperature within narrow limits. The core temperature is regulated by the hypothalamus. Additionally, blood, brain, spinal cord, tissues and skin are interrelated. The hypothalamus is the dominant thermoregulator in pigs (Campos *et al*., 2017). Thus, skin, blood, surface, spinal cord and non-hypothalamic portions contribute only 20% of the regulatory control (Kurz, 2008). Receptors for warm temperatures are located throughout the body and terminals are part of the spinal cord and central nervous system reaching lastly to the hypothalamus (Kurz, 2008).

Pigs can detect high temperatures from peripheral thermoreceptors to the central nervous system. Thus, during warm temperatures, the preoptic region of the hypothalamus activates mechanisms to dissipate extra heat (Campos *et al*., 2017).

Heat stress depends on the extent of diurnal fluctuations in the temperature. In cows for example, if the temperature drops at night below 21ºC, the animal has enough time to dissipate all the heat gained during the day (Silanikove, 2000).

Activation of the hypothalamic-pituitary-adrenal (HPA) axis corresponds to increased cortisol levels in stressed pig (Campos *et al*., 2017). Thus, high levels of cortisol increase the energy availability to the animal to face the stressful factor (Campos *et al*., 2017). But, under heat stress conditions the available energy due to cortisol start to be dissipated, thus the pig use different

mechanisms for heat dissipation such as panting, reduction in physical activity, reduction in feed intake and change in water consumption. When the heat is dissipated, the concentration of cortisol is reduced and exerts a negative feedback to the hypothalamus; therefore, the body recovers homeostasis (Binsiya *et al*., 2017).

## OXIDATIVE STRESS

### *Oxidative and heat stress*

Oxidants or free radicals are produced by the respiratory chain of mitochondria within the cell (Buchet *et al*., 2017). When immune activation or stress occurs, free radicals are released which can exceed the maximum antioxidant capacity, generating oxidative stress. The oxidative products are accumulated in blood (Buchet *et al*., 2017). Oxidative stress is referred to as an imbalance of free radicals and antioxidant capacity, provoking damage to lipids, proteins and DNA of cell structures, affecting normal cell functioning (Lykkesfeldt and Svendsen, 2007).

Heat stress inducess oxidative stress in intestinal cells compromising the epithelial barrier. As aforementioned, pigs under heat stress face hyperthermia, hypoxia and GI inflammation (Pearce *et al*., 2013a), triggering oxidative stress (Liu *et al*., 2018). Oxidative stress can affect tight junctions and compromise intestinal health due to alteration of redox balance. Disruption of tight junctions allows passage of endotoxins and pathogens such as E. coli Also, differentiation of enterocytes from crypts to villus is negatively affected by heat stress (Vergauwen *et al*., 2015).

### *Reactive oxygen species (ROS)*

Reactive oxygen species (ROS) are produced within mitochondria when the final oxygen electron receptor is reduced to a molecule of water and electrons leak out of the system prematurely, producing ROS (Lykkesfeldt and Svendsen, 2007; Vergauwen *et al*., 2015). The first radical formed is the superoxide anion ($O_2^-$) in the electron transport chain when $O_2$ get a single

electron. ROS types include superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals (·OH) (Shi-bin *et al*., 2007), with ·OH being the most reactive free radical (Lykkesfeldt and Svendsen, 2007).

Normally ROS act to destroy bacteria and cells. High ROS concentrations can cause damage to proteins (provoking malfunctioning of enzymes), lipid (damaging lipid bilayer cellular membranes, causing oxidation damage) and DNA (causing mutations and possible cell death) (Lykkesfeldt and Svendsen, 2007). There are numerous stress factors that produce ROS within peroxisomes and mitochondria, such as xanthine oxidase, NO synthetase, NADPH oxidases, radiation UV and heat (Brieger *et al*., 2012 and Vergauwen *et al*., 2015).

ROS have the following functions: redox regulation of phosphorylation of proteins and ion channels. ROS can be hydrogen peroxide, superoxide anion, and hydroxyl radicals, which are very reactive and can react with almost all biological molecules (Vergauwen *et al*., 2015).

In an *in vitro* study conducted by Vergauwen *et al*. (2015), cells pre-treated with vitamin E had reduced intracellular oxidative stress when induced by hydrogen peroxide ($H_2O_2$). In the same study, $H_2O_2$ increased cellular membrane permeability. Zona occluden-1 (ZO-1) is a tight junction protein associated with oxidative stress (Vergauwen *et al*., 2015). Vitamin E pre-treated cells increased regenerative capacities when compared with untreated cells (Vergauwen *et al*., 2015).

***Natural antioxidant system***

The body has a natural antioxidant system, which serves as a defense mechanism against ROS and oxidative stress, such as antioxidant enzymes and antioxidant scavengers. The antioxidant system is composed of intrinsic sources, including glutathione, superoxide dismutase and catalase. It also can include external sources such as vitamin A, C and E and selenium (Buchet

*et al*., 2017). Vitamin C and E and glutathione are low molecular weight antioxidants. Enzymatic antioxidants are high molecular weight antioxidants (i.e., superoxide dismutase) (Lykkesfeldt and Svendsen, 2007).

Production of ROS and the antioxidant system normally is in balance in the body (Shi-bin *et al*., 2007). When antioxidants donate electrons to oxidants, they themselves become radicals but are stable such that they are not able to induce cellular damage. The oxidized antioxidants are recycled and regenerated to their active state by NADPH, which provides energy for this process. Thus, the antioxidant system is renovated each time (Lykkesfeldt and Svendsen, 2007). Glutathione and ascorbate are antioxidant biomarkers because they recycle antioxidants to the initial active state (Lykkesfeldt and Svendsen, 2007). Cells have their own mechanism of defense against increased ROS in the body as described in the Figure 3.

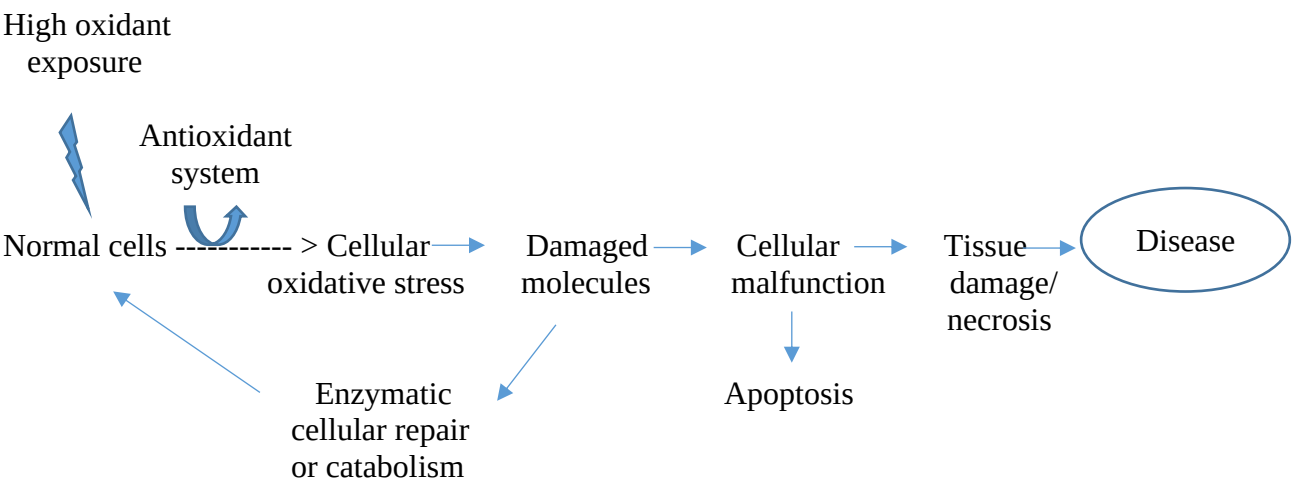


**Figure 3.** Cellular defense against high oxidant exposure to normal cells. Adapted from (Lykkesfeldt and Svendsen, 2007). High oxidant exposure in normal cells is counteracted by the natural antioxidant system allowing the return to cellular homeostasis. Cellular oxidative stress causes some damage in proteins, lipids and DNA, which can be repaired or catabolized by enzymes. Cellular malfunction generates controlled cell death (apoptosis). If not controlled, it can cause tissue damage and uncontrolled cell death (necrosis) provoking disease.

The antioxidant superoxide dismutase is an enzyme which catalyze the chemical reaction of superoxide ($O_2^-$) into oxygen ($O_2$) and $H_2O_2$ Then, catalase enzyme converts $H_2O_2$ to $H_2O$. Moreover, glutathione synthetase originates peroxidase which reduces $H_2O_2$ to water and reduces lipid hydroperoxides to alcohols (Brieger *et al*., 2012). As aforementioned, antioxidant scavengers such as vitamin E, ascorbic acid, carotenoids and polyphenols are added into diets as exogenous sources to reduce oxidative stress (Brieger *et al*., 2012).

There are commercial assays to determine the activity of all antioxidants in a value (i.e., total antioxidant status and Trolox equivalent antioxidant capacity) and to determine DNA damage (8-hydroxydeoxyguanosine-8OHdG), protein oxidation (protein carbonyls) and lipid peroxidation (malondialdehyde; MDA) (Lykkesfeldt and Svendsen, 2007).

## LIPID PEROXIDATION

### *Lipids and lipid peroxidation*

Lipids containing polyunsaturated fatty acids (PUFA) are prone to oxidation (Koch *et al*., 2017) and are susceptible to free radical damage. When lipids are heated with $O_2$, peroxides and then hydroperoxides are produced. Lipid peroxidation occurs when the primary lipoxyl radical is unstable and produces secondary products such as aldehydes, ketones, hydrocarbonyls and carbonyls in the presence of metals. Lipid peroxidation is a parameter that indicates the extent of oxidative stress present in cells (Zhang *et al*., 2015).

Peroxidation causes generation of genotoxic and cytotoxic compounds, including 4-hydroxy-2-alkenals (4-HHE) and 4-hydroxy-2-nonenal (4-HNE) as principal end products from *n*-3 and *n*-6 polyunsaturated fatty acid (PUFA) oxidation (Awada *et al*., 2012). According to Awada *et al*. (2012), the concentrations of 4-HHE were high in plasma of rats when compared with non-oxidized oil. This fact shows the damage caused to cells by lipid peroxidation. Lipid peroxidation

causes inflammation in the intestine. Thus, the number of Paneth cells (involved in intestinal protection) in the duodenum were reduced and nuclear factor kappaB (NF-κB) was activated due to lipid peroxidation. Likewise, cells of rats incubated with 4-HHE and 4-HNE had increases in carbonyl groups derivatives from protein carbonylation when compared with untreated cells (Awada *et al*. (2012).

Thus, biomarkers of lipid peroxidation are considered good indicators of oxidative stress. One of them is malondialdehyde (MDA), which comprises products from decomposition of PUFA due to peroxidation (Celi, 2011 and Shi-bin *et al*., 2007, Zhang *et al*., 2015). High concentrations of MDA were reported in piglets fed with 5% of peroxidized fish oil in the diets. Likewise, the piglets had low growth performance due to oxidative stress damaging the antioxidant system of the piglets (Shi-bin *et al*., 2007).

Thiobarbituric acid reactive substances (TBARS) provide an indication of peroxidation in lipid and was tested in muscle, indicating lower concentrations in muscle of pigs fed high vitamin E concentration in feed (Kim *et al*., 2015). There are natural antioxidants alternatives such as vitamin E, polyphenols and phytogenics to reduce the impact of lipid peroxidation and oxidative stress in animals.

## ANTIOXIDANTS

### *Vitamin E*

Vitamin E is classified as lipid soluble antioxidant and can reduce free radical concentrations in cells (Liu *et al*., 2018). Vitamin E cannot be synthetized and dietary supplementation is required (Wang *et al*., 1996). Vitamin E is classified into 4 natural tocopherols bearing a saturated phytyl $C_{16}$ side chain (α, β, γ, and δ) and 4 tocotrienols bearing three double bonds in the phytyl side chain (α, β, γ, and δ) (Bjørneboe *et al*., 1990). A molecule of vitamin E

includes two parts, a hydroxyl-bearing aromatic system (responsible for antioxidant properties) and a saturated (tocopherol) or polyunsaturated (tocotrienol) hydrocarbon tail that orients the vitamin E molecule in the lipid membrane (Lúcio *et al*., 2009).

The term tocopherol is derived from the Greek term “tokos” and “pherein” meaning to bring forth childbirth (Bjørneboe *et al*., 1990). α-Tocopherol has the highest biologically activity and is the predominant form of vitamin E in many species (Bjørneboe *et al*., 1990) (Figure 4). The active site of the tocopherol molecule is the 6-hydroxyl group, and the side chain in the 2-position allows incorporation and retention of the vitamin E in biological membranes (Bjørneboe *et al*., 1990).

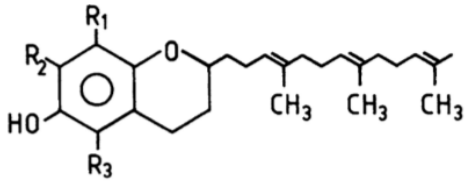


**Figure 4.** Tocopherol structure. Adapted from (Bjørneboe *et al*., 1990).

The d isomer has more reactivity than the l isomer. Thus d-α-tocopherol has more biological activity than the l isomer of vitamin E (Liu *et al*., 2018). Seed and plants are natural sources of vitamin E. Part of vitamin E is lost during storage under heat and moisture, and exposure to fat and trace minerals of feed ingredients (Wilburn *et al*., 2008, NRC, 2012 and Wang *et al*., 1996). Synthetic vitamin E contain 8 isomers (all-rac-α-tocopherol) equally proportioned, each with different biological activity. In practical nutrition, synthetic vitamin E (dl-α-tocopheryl acetate) is commonly used due to reduced cost compared to natural vitamin E (Wilburn *et al*., 2008).

According to NRC. (2012), 1 IU vitamin E is equivalent to 0.67 mg of d-α-tocopherol or 1 mg of dl-α-tocopheryl acetate, thus d-α-tocopherol as natural source of vitamin E is more active

than synthetic dl-α-tocopheryl acetate. This fact was reported by Wilburn *et al*. (2008) when comparing natural with synthetic vitamin E in a pig trial. Requirements of vitamin E for piglets of 5 to 11 kg BW is 16 IU vitamin E/kg of feed and from 11 to 135 kg BW, it is 11 IU of vitamin E/ kg of feed (NRC, 2012).

Vitamin E is absorbed primarily in the medium part of the small intestine. No differences were reported in the absorption of tocopherol and tocotrienols. In addition, high amounts of vitamin E intake leads to lower absorption efficiencies, resulting in high amounts of vitamin E in feces (Combs and McClung, 2017).

Vitamin E as a lipid soluble vitamin is absorbed by the intestine by action of lipases and bile acids depending on the adequate absorption of lipids (Combs and McClung, 2017). When lipases and bile acids are decreased during the postweaning period in piglets, digestibility and absorption of vitamin E is decreased (Jensen *et al*., 1997).

In general, the absorption of vitamin E is a micelle-facilitated diffusion process, which requires fat in the gut (lumen) and pancreatic esterases to release free fatty acids from dietary triglycerides, and bile acid to form mixed micelles. The presence of PUFAs interacting with tocopherols in the lumen can produce absorption stimulated by intragastric medium-chain triglycerides and inhibited by linoleic acid (Combs and McClung, 2017).

When vitamin E is absorbed by the intestine it enters to the lymphatic circulation with the triglyceride-rich chylomicrons (Combs and McClung, 2017). Within enterocytes, vitamin E is combined with other lipids, to form chylomicrons and very low-density lipoproteins (VLDLs). In pigs, the chylomicrons are released into the lymphatic circulation.

An increase in the consumption of vitamin E is linearly correlated with increased serum or plasma vitamin E concentration in pigs when supplied through the feed (Hasty *et al*., 2002,

Wilburn *et al*., 2008 and Kim *et al*., 2015) and in drinking water (van Heugten *et al*., 1997 and Wilburn *et al*., 2008). At high intake of vitamin E, the relative increase in serum or plasma vitamin E decreases. This is due to an increased uptake of vitamin E by the liver and incorporation into VLDLs produced by liver. Vitamin E does not have a specific carrier protein in plasma and it is transferred from chylomicrons to plasma lipoproteins. Circulating chylomicrons transfer tocopherols to tissue (Combs and McClung, 2017).

Plasma tocopherols are distributed by very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs) being present at higher concentrations in the latter. Thus, α-tocopherol transport by chylomicrons is the most important variable between individuals (Combs and McClung, 2017). Absorption and transport of vitamin E are described in Figure 5.

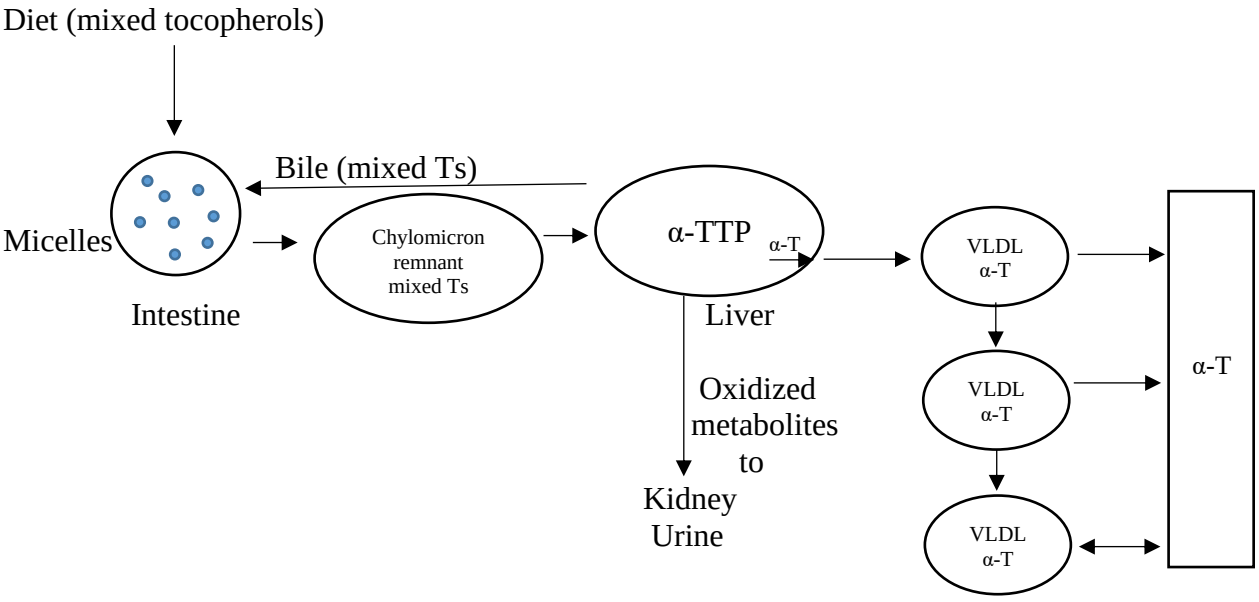


**Figure 5.** Absorption and transport of vitamin E in the body. (Adapted from Combs and McClung, 2017). α-T: α-tocopherol, mixed Ts: mixed tocopherols and α-TTP: α-tocopherol transfer protein.

Retention of vitamin E in muscle after addition of 35 and 300 IU in feed was increased (Kim *et al*., 2015). High tissue concentrations of vitamin E are found in adipose tissue as the principal location, followed by liver, heart, loin and ultimately in kidneys (Wang *et al*., 1996 and Wilburn *et al*., 2008). Vitamin E takes about 14 to 21 days to cause differences in plasma concentration in piglets when fed the vitamin in feed (Wang *et al*., 1996). On the other hand, van Heugten *et al*. (1997) showed vitamin E concentrations increased in serum on day 1 and day 7 after weaning of supplementation of vitamin E in drinking water when compared with initial concentrations. This fact supports the statement that vitamin E in drinking water is more effective to saturate the pig body than dietary supplementation. Wilburn *et al*. (2008) and Amazan *et al*. (2014) also reported higher concentrations of vitamin E after adding vitamin E in drinking water than in feed in postweaning piglets. Authors reported no effects on ADG or ADFI for any postweaning period when vitamin E was supplemented in feed or drinking water (Wilburn *et al*., 2008). van Heugten *et al*. (1997) also reported no differences in gain-to-feed ratio between control and treated pigs during 2 weeks with vitamin E and selenium supplementation, but for 5 weeks supplementation treated pigs had higher gain-to-feed ratio than control pigs (van Heugten *et al*., 1997).

Piglets during the initial two days postweaning period present low levels of vitamin E (α-tocopherol) in tissue and plasma. This fact is due to stress postweaning and low feed intake triggering low vitamin E amounts ingested (Wang *et al*., 1996). On the other hand, Wilburn *et al*. (2008) reported high concentration of vitamin E in serum from 0 to day 3 postweaning when vitamin E was supplemented in drinking water or with 300 IU dietary vitamin E/kg of feed.

Deficiency of vitamin E in pigs can cause muscle degeneration (cardiac and skeletal), parakeratosis, liver necrosis and death. Vitamin E toxicity has not been reported in pigs (NRC,

2012). However, vitamin E supplementation at high doses had negative effects in humans. As reported by Miller *et al*. (2005), vitamin E supplementation in excess in humans increased α-tocopheroxyl radicals and may displace other fat-soluble vitamins, such as γ-tocopherol, producing a disruption in the equilibrium between oxidants and the natural antioxidant system.

***Impact of Vitamin E on heat and oxidative stress conditions in pigs***

In a study reported by Liu *et al*. (2018), the addition of 200 IU of vitamin E/kg of feed did not alleviate oxidative stress in growing pigs under heat stress and did not impact rectal temperature and respiration rate. Zou *et al*. (2016) reported that vitamin E supplementation showed less efficacy in growing pigs when compared with essential oils in feed during transport stress.

Piglets after weaning were observed to have decreased concentrations of α-tocopherol during 14 days after weaning, and these decreases were higher in piglets that had high serum concentration of α-tocopherol at weaning (Amazan *et al*., 2014). Oxidative stress markers such as malondialdehyde (MDA) in serum was increased in piglets when supplemented with vitamin E in feed compared to drinking water, and levels of MDA were higher at low inclusion of vitamin E in drinking water (Amazan *et al*., 2014).

***Impact of Vitamin E on lipid peroxidation***

Vitamin E as fat-soluble vitamin is lipophilic in nature and protects the membrane phospholipids from peroxidation reactions (Tengerdy, 1990, Wang *et al*., 1996 and Lúcio *et al*., 2009). Thus, the vitamin E hydroxyl group is located close to the membrane surface for scavenging purposes (Lúcio *et al*., 2009). Unsaturated lipids are more susceptible to be affected by free radicals, especially polyunsaturated fatty acids due to their unsaturated double bonds being unstable (Wang *et al*., 1996). Additionally, the effect of vitamin E depends also on the

presence of other antioxidants. For each increased gram of PUFA in the diet, 1 to 3 mg of vitamin E is needed to compensate (Tengerdy 1990).

Cell membranes are composed of phospholipid bilayers where free radicals attack, producing lipid peroxidation. If not rapidly quenched by the antioxidant system, cytotoxic compounds can be produced, disrupting important biological molecules such as proteins and DNA (Lúcio *et al*., 2009).

Lipid peroxidation is described as the abstraction of a hydrogen atom from a PUFA, leaving an electron on the carbon atom. This lipid radical may interact with oxygen molecules or any other oxygen-derived molecule, producing a hydroperoxide radical. The hydroperoxide radical abstract hydrogen atoms from other lipids and continues the reaction, producing various products. Thus, vitamin E could terminate this chain reaction which allows free radical scavenging and end lipid peroxidation (Bjørneboe *et al*., 1990 and Lúcio *et al*., 2009) (Figure 6). The vitamin E radical is not stable due to the unpaired electron on the oxygen in the C-6 position, but it can be delocalized into the aromatic ring, increasing its stability. Cells exposed to oxygen molecules can be damaged by free radicals and lipid peroxidation products (Bjørneboe *et al*., 1990). Description is shown on Figure 6.

When protection from vitamin E fails, lipid peroxidation chain reactions can occur, disrupting normal membrane functioning. One of the products of this reaction is the presence of malondialdehyde (MDA) (Wang *et al*., 1996).

High oxidant diets containing 5% oxidized soybean oil and 10% PUFA decreased growth performance and increased protein carbonyl (an oxidative stress marker) and TBARS in plasma and liver in growing-finishing pigs (Lu *et al*., 2014b). Additionally, the same authors reported that supplementation of the combination of an antioxidant blend with vitamin E in the diet for piglets,

containing 5% of peroxidized soybean oil and 10% PUFA, improved growth performance, liver function, and reduced plasma oxidative markers.

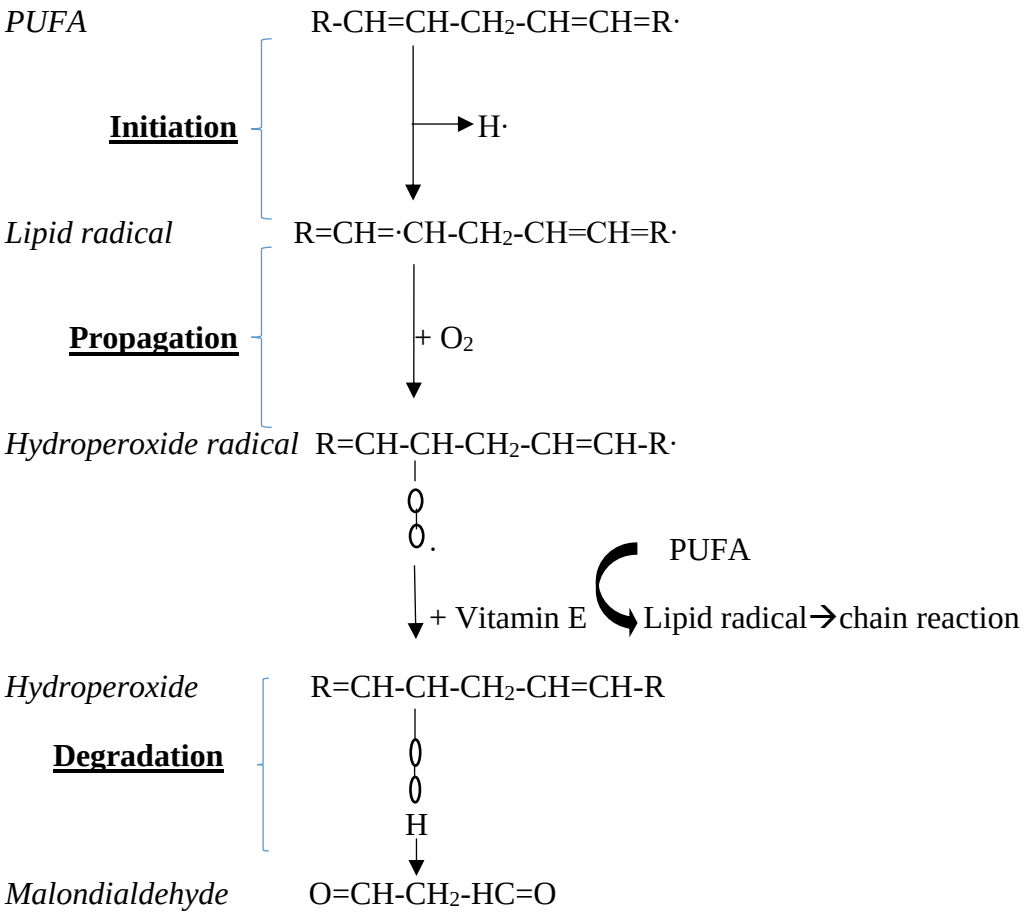


**Figure 6.** Lipid peroxidation reaction. Adapted from (Bjørneboe *et al*., 1990).

***Polyphenols***

The term "phenolic" refers to compounds containing an aromatic ring connecting one hydroxyl group, thus "polyphenols" can have one or more than one aromatic ring connecting hydroxyl groups (Zhang and Tsao, 2016). Phenolic compounds or polyphenols are distributed in fruits, vegetables, grains, essential oils, herbs, and wine (Lipinski *et al*., 2017). They provide protection mechanisms against biotic (insects or microorganisms) and abiotic stresses (Zhang and

Tsao, 2016). This property can affect its absorption in humans. When a plant is invaded by insects, the polyphenols glucosides and aglycones are released, varying in their absorption because glycosides are less well absorbed than aglycones. Flavonoids are present in plants as glycosides or aglycones in plants and are responsible of coloration (Lipinski *et al*., 2017).

Polyphenols are absorbed at approximately 5-10% in the upper gastrointestinal tract and their availability is lower than antioxidant vitamins because of rapid metabolism (Zhang and Tsao, 2016). This fact affects their detection in vivo. (Zhang and Tsao, 2016). In the gastrointestinal tract and colon, polyphenols are depolymerized and deconjugated, then the products undergo liver phase I and II metabolism, being absorbed in the systemic circulation and tissues to act as antioxidants or they are excreted through urine after glucuronidation, sulphation and O-methylation (Zhang and Tsao, 2016). The remaining unabsorbed polyphenols are released by the colonic microorganisms producing anti-inflammatory or prebiotic functions *in situ*. Polyphenols do not accumulate at high concentrations in the body; they accumulate only in organs that participate in their metabolism (Lipinski *et al*., 2017).

Polyphenols derived from grape improved growth performance in weaned piglets (Fiesel *et al*, 2014), even though plasma vitamin E concentration and antioxidative capacity were not altered by weaning (Gessner *et al*., 2013). There is not enough evidence to suggest that polyphenols can replace vitamin E as an antioxidant. Polyphenols can help vitamin E to be more effectively recycled or diminish free radicals (Surai, 2014).

In general, polyphenols have anti-inflammatory, antiallergic, immunomodulatory and antioxidant benefits (Lipinski *et al*., 2017). Polyphenols have these antioxidant and anti-inflammatory properties due to their chemical structures; it is the aromatic and multiple hydroxyl group features enabling donation of electrons or hydrogen atoms to free radicals and ROS,

stopping oxidation reactions (Zhang and Tsao, 2016). Likewise, supplementation of polyphenols caused a reduction in pro-inflammatory cytokines such as TNF-α, IL-8 and improvements in small intestine morphology in piglets (Gessner *et al*., 2013).

According to Gerasopoulos *et al*. (2015) oxidative stress markers such as total antioxidant capacity (TAC) and reduced glutathione (GSH) were present at high concentrations in tissues (brain, heart, kidneys, liver, and lungs) of pigs of 50 days old when fed polyphenols-supplemented diets in comparison with non-polyphenols supplemented diet. Additionally, TBARS concentration decreased in the same tissues when using polyphenols in the diet. In contrast, Gessner *et al*. (2013) showed no change in plasma TBARS concentrations in weaned piglets with supplementation of polyphenols. Polyphenols and vitamin E have strong antioxidant potential, but polyphenols did not influence vitamin E status in pigs (Gessner *et al*., 2013).

Is well known that weaning of piglets reduces antioxidant mechanisms (Gerasopoulos *et al*., 2015) by the interaction of natural antioxidant system represented by catalase and superoxide dismutase and weaning stressor factors (changes on environment, social and feeding). Therefore, the supplementation of polyphenols in the diet for piglets improves gain:feed ratio as was shown by Fiesel *et al*. (2014).

***Impact of polyphenols on heat and oxidative stress conditions in pigs***

When the body is exposed to chemical stress, redox imbalance and lack of oxygen, these can cause acute or chronic inflammation. Thus, in acute inflammation, cytokines are released and ROS, such as hydroxyl radical and superoxide anion in high concentrations affect the immune equilibrium in the body (Zhang and Tsao, 2016). Dietary polyphenols neutralize these free radicals donating an electron or hydrogen atom. Polyphenols disrupt lipid autoxidation as radical scavenger or metal chelators converting hydroperoxides into stable compounds (Zhang and Tsao, 2016). The

potential of polyphenols as antioxidant depends on the number and position of its hydroxyl groups. Importantly, when a polyphenol loses an electron it becomes a prooxidant radical, also during high supplementation of polyphenols (Zhang and Tsao, 2016).

Polyphenols have the capacity to restore redox equilibrium and prevent inflammation enhancing activities of antioxidant enzymes such as SOD, CAT, GPx and GR. Erythroid-related factor (Nrf2) can modulate these antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) during the presence of high concentrations of ROS in the mitochondria. Thus, dietary polyphenols can trigger Nrf2, activating antioxidant system to stabilize cellular redox equilibrium (Yang, *et al*., 2015 and Zhang and Tsao, 2016).

Overproduction of ROS promotes synthesis of pro-inflammatory cytokines, hence, polyphenols inhibit oxidative stress, reducing inflammation via NFκB and mitogen-activated protein kinase (MAPK). Thus, pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IFN-γ and TNF-α, are released in the bloodstream disrupting immune equilibrium when not regulated (Zhang and Tsao, 2016). Pyrin domain-containing 3 (NLRP3) crosslink the signaling between redox response and inflammation. IL-1β induces activation of Toll-like receptor-1 (TLR-1) triggering NFκB and MAPK triggering a systemic inflammation. (Zhang and Tsao, 2016).

Flavonoids can inhibit IL-1β secretion and reduce inflammation by inhibiting the activity of 5-lipoxygenase (5-LOX) and cyclooxygenase (COX) (Lipinski *et al*., 2017). At the same time, resveratrol (polyphenol found in grapes) inhibits NLRP3 reducing ROS in cells (Zhang and Tsao, 2016). Supplementation of polyphenols inhibits NFκB decreasing expression of pro-inflammatory cytokines such as IL-1β, IL-8 and TNF in the intestine of pigs (Fiesel *et al*., 2014).

## Phytogenics

Phytogenics are feed additives derived from plants that are used in animal feeding programs. They have antioxidant (Cuppert and Hall, 1998), antimicrobial (Yang *et al*., 2015) and anti-inflammatory properties. These properties improve feed intake and consequently growth performance (Windisch *et al*., 2008 and Yang *et al*., 2015). Phytogenics are considered herbs, spices, and essential oils. The variation in phytogenics compounds is associated with the part of the plant that is used, harvest season and geographical origin (Windish *et al*., 2008).

As aforementioned, phytogenics have antioxidant properties, protecting cells from oxidative stress. Essential oils are a major group of phytogenics (Yang *et al*., 2015). Ranking phytogenics for antioxidant capacity from high to low effectivity is as follows for essential oils: jasmine oil, rose oil, ylang-ylang oil, celery oil and angelica seed oil (Wei and Shibamoto, 2007). Phytogenics have been shown to improve palatability of the diets for animals due to flavor and odor properties (Yang *et al*., 2015).

### *Impact of phytogenics on oxidative stress and lipid peroxidation in pigs*

Essential oils are part of plants and serve as insect attractant for pollination processes. They also serve as a way against insect attacks (Cuppett and Hall, 1998). The antioxidant activity of essential oils can vary depending on their composition and concentration; i.e., clove is a more potent antioxidant than thyme (Cuppert and Hall, 1998). The antioxidant activity of essential oils is due to the presence of a hydroxyl group on the aromatic ring, thus, essential oils such as eugenol and thymol are more potent antioxidants than carvone, thujone and borneol, which do not have a hydroxyl group in their structure (Cuppett and Hall, 1998) as shown in Figure 7.

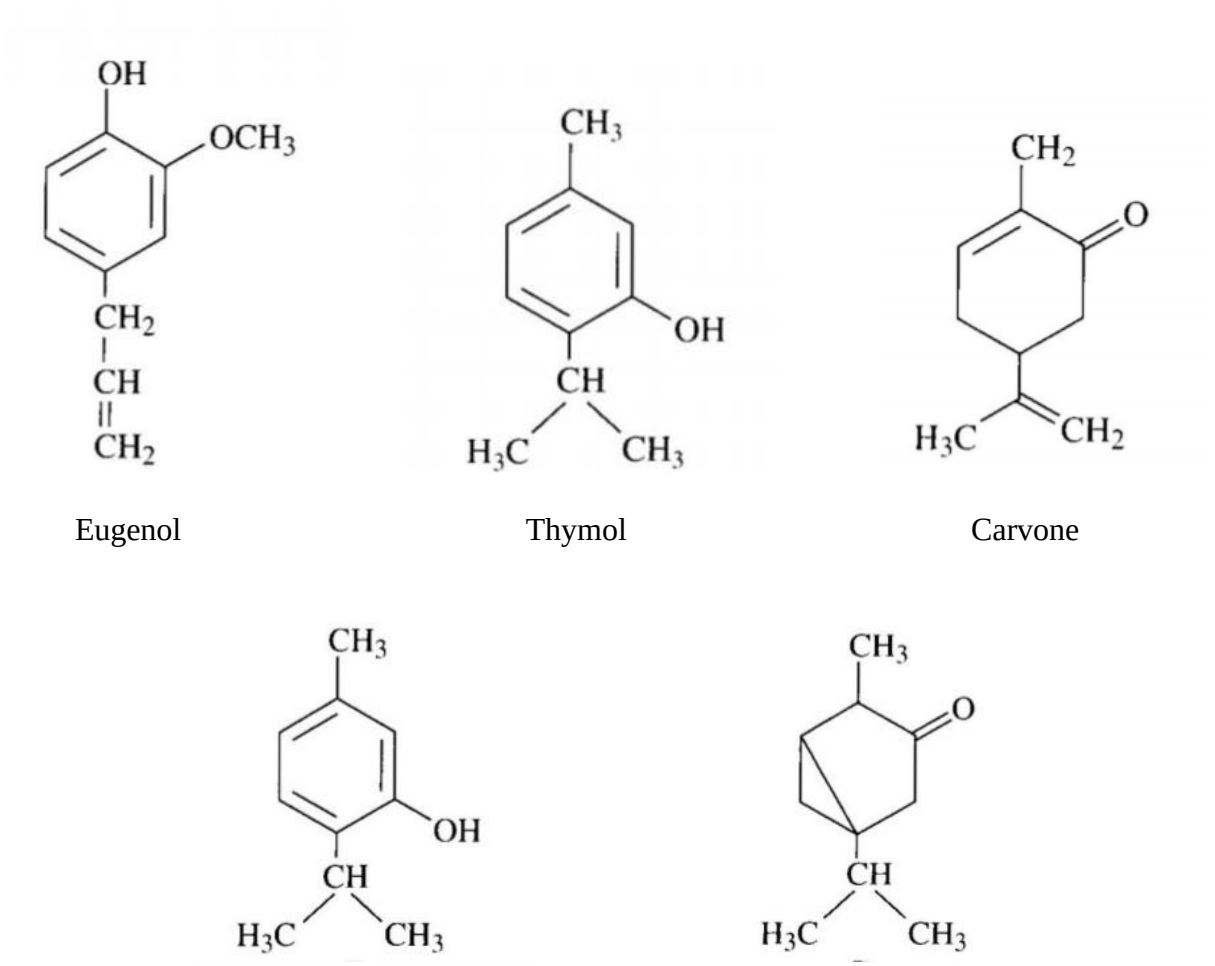


**Figure 7.** Different structures of essential oils. Adapted from (Cuppett and Hall, 1998)

Oregano oil serves to alleviate oxidative stress and improving antioxidant activity in growing pigs (Zhang *et al*., 2015). Carvacrol and thymol as principal components of oregano act as phenolic compounds (Zhang *et al*., 2015). Oregano essential oil reduced cortisol, HSP 70 (heat shock protein 70), and recover damage in the intestine epithelium in pigs during transport (Zou *et al*., 2016).

As aforementioned, ROS (oxidative stress marker) and MDA (lipid peroxidation marker) in serum and in liver tissue can be found at higher concentrations in growing pigs under stressed events (Zhang *et al*., 2015). Essential oils such as jasmine, parsley, rose, celery seed, ylang-ylang,

and juniper berry decreased MDA concentrations in skin surface under UV irradiation (Wei and Shibamoto, 2007).

Heat shock proteins are increased during stressed events in pigs (Zhang *et al*., 2015). Oregano oils can act as a free radical scavenging compound, reducing the concentration of MDA in serum of pigs under stressed events (Zhang *et al*., 2015). Antioxidants originating from Labiatae, zingiberaceae (ginger) and allspice (pimenta dioica) have phenolic antioxidant properties that can reduce lipid peroxidation and inflammation in humans (Nakatani, 2000).

Inflammation in the intestine caused by infectious disease, allergy to diet components, and weaning has negative effects on performance in pigs (Yang *et al*., 2015). Nrf2 and NF-κB are involved in the protection from oxidative stress and the reduction of inflammation in the gut in pigs (Gessner *et al*., 2013).

Weaning is a stress factor for piglets, which is associated with gastrointestinal disorders due to overproduction of ROS resulting in oxidative stress (Zhu *et al*., 2012). Additionally, weaning in piglets causes alterations in intestinal morphology, inhibition in the antioxidant system and increased concentrations of MDA and $H_2O_2$. (Zhu *et al*., 2012).

Heat stress and feeding of peroxidized lipids both induce oxidative stress that impacts intestinal health, immune status and growth performance of pigs. According to literature reviewed and presented in this dissertation, there is a relationship between oxidative stress, vitamin E concentrations, and intestinal health during the post-weaning period that may assist in the recovery from this stress. Therefore, there is an opportunity to investigate the effects of the addition of vitamin E and polyphenols in pigs during heat stress or pigs fed lipid peroxidized diets to determine the potential effects of antioxidants during oxidative stress in pigs. Although feed intake may be compromised during the postweaning period, water consumption seems likely not affected by this

factor. Therefore, supplementation of antioxidants in the water may be of particular interest in alleviating stress associated with excess heat or the feeding of diets with peroxidized lipids. Thus, the objective of the current dissertation was to evaluate the effectiveness of antioxidant supplementation in water or feed on performance, oxidative status, and oxidative stress markers in pigs exposed to heat stress or oxidative stress.

**References**

Akbarian, A., Michiels, J., Degroote, J., Majdeddin, M., Golian, A., & De Smet, S. (2016). Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *Journal of Animal Science and Biotechnology, 7(1),* 37.doi:10.1186/s40104-016-0097-5

Amazan, D., Cordero, G., López-Bote, C. J., Lauridsen, C., & Rey, A. I. (2014). Effects of oral micellized natural vitamin E (d-α-tocopherol) v. synthetic vitamin E (dl-α-tocopherol) in feed on α-tocopherol levels, stereoisomer distribution, oxidative stress and the immune response in piglets. *Animal: An International Journal of Animal Bioscience, 8*(3), 410-419. doi:10.1017/S1751731113002401

Awada, M., Soulage, C., Meynier, A., Debard, C., Plaisancie, P., Benoit, B., Picard, G., Loizon, E., Chauvin, M.A., Estienne, M., Peretti, N., Guichardant, M., Lagarde, M. and Michalski, M. (2012). Dietary oxidized n-3 PUFA induce oxidative stress and inflammation: Role of intestinal absorption of 4-HHE and reactivity in intestinal cells. *Journal of Lipid Research, 53*(10), 2069-80. doi:10.1194/jlr.M026179

Baumgard, L. H., & Rhoads, R. P. (2013). Effects of heat stress on postabsorptive metabolism and energetics. *Annual Review of Animal Biosciences, 1*(1), 311-337. doi:10.1146/annurev-animal-031412-103644

Bhat, A. A., Uppada, S., Achkar, I. W., Hashem, S., Yadav, S. K., Shanmugakonar, M., Al-Naemi, H. A., Haris, M. and Uddin, S. (2018). Tight junction proteins and signaling pathways in cancer and inflammation: A functional crosstalk. *Frontiers in Physiology, 9,* 1942. doi: 10.3389/fphys.2018.01942

Binsiya T. K, Sejian V., Bagath M., Krishnan G., Hyder I., Manimaran A., Lees A.M., Gaughan J.B., and Bhatta R. (2017). Significance of hypothalamic-pituitary-adrenal axis to adapt to climate change in livestock. International Research Journal of Agricultural and Food Sciences 2, 1–20.

Bjørneboe, A., Bjørneboe, G. E., & Drevon, C. A. (1990). Absorption, transport and distribution of vitamin E. *The Journal of Nutrition, 120*(3), 233-242. doi:10.1093/jn/120.3.233

Brenes, A., Viveros, A., Goñi, I., Centeno, C., Sáyago-Ayerdy, S. G., Arija, I., & Saura-Calixto, F. (2008). Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poultry Science, 87*(2), 307-316. doi:10.3382/ps.2007-00297

Brieger, K., Schiavone, S., Miller, J., F J, & Krause, K. (2012). Reactive oxygen species: From health to disease. *Swiss Medical Weekly, 142*, 13659. doi:10.4414/smw.2012.13659

Buchet, A., Belloc, C., Leblanc-Maridor, M., & Merlot, E. (2017). Effects of age and weaning conditions on blood indicators of oxidative status in pigs. *PLoS One, 12*(5), e0178487. doi:10.1371/journal.pone.0178487

Buettner, G. R. (1993). The pecking order of free radicals and antioxidants: Lipid peroxidation, alpha-tocopherol, and ascorbate. *Archives of Biochemistry and Biophysics, 300*(2), 535-543. doi:10.1006/abbi.1993.1074

Campos, P. H. R. F., Le Floc'h, N., Noblet, J., & Renaudeau, D. (2017). Physiological responses of growing pigs to high ambient temperature and/or inflammatory challenges. *Revista Brasileira De Zootecnia, 46*(6), 537-544. doi:10.1590/s1806-92902017000600009

Campos, P., Noblet, J., Jaguelin-Peyraud, Y., Gilbert, H., Mormède, P., de Oliveira Donzele, R., Donzele, J. L., and Renaudeau, D. (2014). Thermoregulatory responses during thermal acclimation in pigs divergently selected for residual feed intake. *International Journal of Biometeorology, 58*(7), 1545-1557. doi:10.1007/s00484-013-0759-3

Celi, P. (2011). Biomarkers of oxidative stress in ruminant medicine. *Immunopharmacology and Immunotoxicology, 33*(2), 233-240. doi:10.3109/08923973.2010.514917

Collin, A., van Milgen, J., Dubois, S., & Noblet, J. (2001). Effect of high temperature and feeding level on energy utilization in piglets. *Journal of Animal Science, 79*(7), 1849. doi:10.2527/2001.7971849x

Collin, A., Vaz, M., & Le Dividich, J. (2002). Effects of high temperature on body temperature and hormonal adjustments in piglets. *Reproduction, Nutrition, Development, 42*(1), 45-53. doi:2002005

Combs, G. F., & McClung, J. P. (2017). In Combs G. F., McClung J. P.(Eds.), *Chapter 8 - vitamin E* Academic Press. doi.org/10.1016/B978-0-12-802965-7.00008-3

Cuppett, S. L., & Hall, C. A. (1998). In Taylor S. L. (Ed.), *Antioxidant activity of the labiatae* Academic Press. doi.org/10.1016/S1043-4526(08)60097-2

Dokladny, K., Moseley, P. L., & Ma, T. Y. (2006). Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. *The American Journal of Physiology, 290*(2), G204. doi.org/10.1152/ajpgi.00401.2005

Ebrahimzadeh, S. K., Navidshad, B., Farhoomand, P., & Mirzaei Aghjehgheshlagh, F. (2018). Effects of grape pomace and vitamin E on performance, antioxidant status, immune response,

gut morphology and histopathological responses in broiler chickens. *South African Journal of Animal Science, 48*(2), 324-336. doi:10.4314/sajas.v48i2.13

Fiesel, A., Gessner, D. K., Most, E., & Eder, K. (2014). Effects of dietary polyphenol-rich plant products from grape or hop on pro-inflammatory gene expression in the intestine, nutrient digestibility and faecal microbiota of weaned pigs. *BMC Veterinary Research, 10*(1), 196. doi:10.1186/s12917-014-0196-5

Gabler, N. K., Koltes, D., Schaumberger, S., Murugesan, G. R., & Reisinger, N. (2018). Diurnal heat stress reduces pig intestinal integrity and increases endotoxin translocation. *Translational Animal Science, 2*(1), 1-10. doi:10.1093/tas/txx003

Gaffin, S. L., & Hubbard, R. (1996). Experimental approaches to therapy and prophylaxis for heat stress and heatstroke. *Wilderness & Environmental Medicine, 7*(4), 312-334. doi.org/10.1580/1080-6032(1996)007[0312:EATTAP]2.3.CO;2

Gerasopoulos, K., Stagos, D., Petrotos, K., Kokkas, S., Kantas, D., Goulas, P., & Kouretas, D. (2015). Feed supplemented with polyphenolic byproduct from olive mill wastewater processing improves the redox status in blood and tissues of piglets. *Food and Chemical Toxicology, 86*, 319-327. doi:10.1016/j.fct.2015.11.007

Gessner, D. K., Fiesel, A., Most, E., Dinges, J., Wen, G., Ringseis, R., & Eder, K. (2013). Supplementation of a grape seed and grape marc meal extract decreases activities of the oxidative stress-responsive transcription factors NF-κB and Nrf2 in the duodenal mucosa of pigs. *Acta Veterinaria Scandinavica, 55*(1), 18. doi:10.1186/1751-0147-55-18

Girotti, A. W. (1998). Lipid hydroperoxide generation, turnover, and effector action in biological systems. *Journal of lipid research, 39*(8), 1529-1542

Hasty, J. L., van Heugten, E., See, M. T., & Larick, D. K. (2002). Effect of vitamin E on improving fresh pork quality in berkshire- and hampshire-sired pigs1. *Journal of Animal Science, 80*(12), 3230-3237. doi:10.2527/2002.80123230x

Heidemann, S., & Glibetic, M. (2005). Heat stress protects against lung injury in the neutropenic, endotoxemic rat. *Inflammation, 29*(1), 47-53. doi:10.1007/s10753-006-8969-4

IPCC. Hoegh-Guldberg, O., Jacob, D., & Taylor, M. (2019). Chapter 3: Impacts of 1.5°C of Global Warming on Natural and Human Systems.

Jensen, M. S., Jensen, S. K., Jakobsen, K. (1997). Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas, *Journal of Animal Science,* Volume 75, Issue 2, February 1997, Pages 437–445 doi.org/10.2527/1997.752437x

Ji, J., Gu, Z., Li, H., Su, L., & Liu, Z. (2018). Cryptdin-2 predicts intestinal injury during heatstroke in mice. *International Journal of Molecular Medicine, 41*(1), 137-146. doi:10.3892/ijmm.2017.3229

Johnson, J. S., Sanz Fernandez, M. V., Gutierrez, N. A., Patience, J. F., Ross, J. W., Gabler, N. K., Lucy, M. C., Safranski, T. J., Rhoads, R. P., and Baumgard, L. H. (2015b). Effects of in utero heat stress on postnatal body composition in pigs: I. growing phase. *Journal of Animal Science, 93*(1), 71-81. doi:10.2527/jas.2014-8354

Johnson, J. S., Sanz Fernandez, M. V., Patience, J. F., Ross, J. W., Gabler, N. K., Lucy, M. C. Baumgard, L. H. (2015a). Effects of in utero heat stress on postnatal body composition in pigs: II. finishing phase. *Journal of Animal Science, 93*(1), 82-92. doi:10.2527/jas.2014-8355

Joshi, S. S., Lesser, T. J., Olsen, J. W., & O'Hara, B. F. (2016). The importance of temperature and thermoregulation for optimal human sleep. *Energy & Buildings, 131,* 153-157. doi:10.1016/j.enbuild.2016.09.020

Kelly, J. R., Kennedy, P. J., Cryan, J. F., Dinan, T. G., Clarke, G., & Hyland, N. P. (2015). Breaking down the barriers: The gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Frontiers in Cellular Neuroscience, 9,* 392. doi:10.3389/fncel.2015.00392

Kerr, B.J.; Kellner, T.A., & Shurson, G. C. (2015). Characteristics of lipids and their feeding value in swine diets. *Journal of Animal Science and Biotechnology, 6*(1), 30. doi:10.1186/s40104-015-0028-x

Kerr, B. J., Yen, J. T., Nienaber, J. A., & Easter, R. A. (2003). Influences of dietary protein level, amino acid supplementation and environmental temperature on performance, body composition, organ weights and total heat production of growing pigs. *Journal of Animal Science, 81*(8), 1998-2007. doi:10.2527/2003.8181998x

Kim, J. C., Jose, C. G., Trezona, M., Moore, K. L., Pluske, J. R., & Mullan, B. P. (2015). Supra-nutritional vitamin E supplementation for 28 days before slaughter maximises muscle vitamin E concentration in finisher pigs. *Meat Science, 110,* 270. doi: 10.1016/j.meatsci.2015.08.007

Koch, R. E., Hill, G. E., & Costantini, D. (2017). An assessment of techniques to manipulate oxidative stress in animals. *Functional Ecology, 31*(1), 9-21. doi:10.1111/1365-2435.12664

Koorts A. M., Levay P. F., Becker P. J., & Viljoen, M. (2011). Pro- and anti-inflammatory cytokines during immune stimulation: Modulation of iron status and red blood cell profile. *Mediators of Inflammation, 2011,* 716301-11. doi:10.1155/2011/716301

Kruse, R., Essén-Gustavsson, B., Fossum, C., & Jensen-Waern, M. (2008). Blood concentrations of the cytokines IL-1beta, IL-6, IL-10, TNF-alpha and IFN-gamma during experimentally induced swine dysentery. *Acta Veterinaria Scandinavica, 50*(1), 32. doi:10.1186/1751-0147-50-32

Kurz, A., M.D. (2008). Physiology of thermoregulation. *Best Practice & Research: Clinical Anaesthesiology, 22*(4), 627-644. doi:10.1016/j.bpa.2008.06.004

Leon, L. R. (2007). Heat stroke and cytokines. *Progress in brain research* (pp. 481-524). Netherlands: Elsevier Science & Technology. doi:10.1016/S0079-6123(06)62024-4

Lin, M., Kao, T., Su, C., & Hsu, S. S. F. (1994). *Interleukin-1β production during the onset of heat stroke in rabbits.* doi.org/10.1016/0304-3940(94)90108-2

Lingaraju, A., Long, T. M., Wang, Y., Austin, J. R., & Turner, J. R. (2015). Conceptual barriers to understanding physical barriers. *Seminars in Cell and Developmental Biology, 42*, 13-21. doi:10.1016/j.semcdb.2015.04.008

Lipiński, K., Mazur, M., Antoszkiewicz, Z., & Purwin, C. (2017). Polyphenols in monogastric nutrition – A review. *Annals of Animal Science, 17*(1), 41-58. doi:10.1515/aoas-2016-0042

Liu, F., Cottrell, J. J., Furness, J. B., Rivera, L. R., Kelly, F. W., Wijesiriwardana, U., Pustovit, R. V., Fothergill, L. J., Bravo, D. M., Celi, P. Leury, B. J., Gabler, N. K., and Dunshea, F. R. (2016). Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. *Experimental Physiology, 101*(7), 801-810. doi:10.1113/EP085746

Liu, Y., Wang, Z., Xie, W., Gu, Z., Xu, Q., & Su, L. (2017). Oxidative stress regulates mitogen-activated protein kinases and c-jun activation involved in heat stress and lipopolysaccharide-induced intestinal epithelial cell apoptosis. *Molecular Medicine Reports, 16*(3), 2579-2587. doi:10.3892/mmr.2017.6859

Liu, F., Celi, P., Chauhan, S. S., Cottrell, J. J., Leury, B. J., & Dunshea, F. R. (2018). A short-term supranutritional vitamin E supplementation alleviated respiratory alkalosis but did not reduce oxidative stress in heat stressed pigs. *Asian-Australasian Journal of Animal Sciences, 31*(2), 263-269. doi:10.5713/ajas.17.0256

Lu, T., Harper, A. F., Dibner, J. J., Scheffler, J. M., Corl, B. A., Estienne, M. J., Zhao, J., and Dalloul, R. A. (2014a). Supplementing antioxidants to pigs fed diets high in oxidants: II. effects on carcass characteristics, meat quality, and fatty acid profile. *Journal of Animal Science, 92*(12), 5464-5475. doi:10.2527/jas.2013-7112

Lu, T., Harper, A. F., Zhao, J., Estienne, M. J., & Dalloul, R. A. (2014b). Supplementing antioxidants to pigs fed diets high in oxidants: I. effects on growth performance, liver function, and oxidative status. *Journal of Animal Science, 92*(12), 5455-5463. doi:10.2527/jas.2013-7109

Lúcio, M., Nunes, C., Gaspar, D., Ferreira, H., Lima, J., & Reis, S. (2009). Antioxidant activity of vitamin E and trolox: Understanding of the factors that govern lipid peroxidation studies in vitro. *Food Biophysics, 4*(4), 312-320. doi:10.1007/s11483-009-9129-4

Lykkesfeldt, J., & Svendsen, O. (2007). Oxidants and antioxidants in disease: Oxidative stress in farm animals. *The Veterinary Journal, 173*(3), 502-511. doi: 10.1016/j.tvjl.2006.06.005

Macari, M., Zuim, S. M. F., Secato, E. R., & Guerreiro, J. (1986). *Effects of ambient temperature and thyroid hormones on food intake by pigs*. doi.org/10.1016/0031-9384(86)90476-2

Mendelsohn, R., & Williams, L. (2004). Comparing forecasts of the global impacts of climate change. *Mitigation and Adaptation Strategies for Global Change, 9*(4), 315-333. doi:MITI.0000038842.35787.1d

Meng, W., & Takeichi, M. (2009). Adherens junction: Molecular architecture and regulation. *Cold Spring Harbor Perspectives in Biology, 1*(6), a002899. doi:10.1101/cshperspect.a002899

Miller, E. R., Pastor-Barriuso, R., Dalal, D., Riemersma, R. A., Appel, L. J. and Guallar, E. (2005). Meta-analysis: High-dosage vitamin E supplementation may increase all-cause mortality. *Annals of Internal Medicine, 142*(1), 37-46. doi:10.7326/0003-4819-142-1-200501040-00110

Montilla, S. I. R., Johnson, T. P., Pearce, S. C., Gardan-Salmon, D., Gabler, N. K., Ross, J. W., Rhoads, R. P., Baumgard, L. H., Lonergan, S. M., Selsby, J. T. (2014). Heat stress causes oxidative stress but not inflammatory signaling in porcine skeletal muscle. *Temperature, 1*(1), 42-50. doi:10.4161/temp.28844

Mount, L. E. (1975). The assessment of thermal environment in relation to pig production. *Livestock Production Science. 2* (1975) 281-292. doi:10.1016/0301-6226(75)90121-9

Mount, L. E. (1978). Heat transfer between animal and environment. *The Proceedings of the Nutrition Society, 37*(1), 21-27. doi:10.1079/PNS19780005

Mullur, R., Liu, Y. & Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiological Reviews, 94*(2), 355-382. doi:10.1152/physrev.00030.2013

Nakatani, N. (2000). Phenolic antioxidants from herbs and spices. *BioFactors, 13*(1-4), 141-146. doi:10.1002/biof.5520130123

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Pandey, K.B., & Rizvi, S.I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity, 2*(5), 270-278. doi:10.4161/oxim.2.5.9498

Pearce, S. C., Mani, V., Weber, T. E., Rhoads, R. P., Patience, J. F., Baumgard, L. H., & Gabler, N. K. (2013a). Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs1. *Journal of Animal Science, 91*(11), 5183-5193. doi:10.2527/jas.2013-6759

Pearce, S. C., Mani, V., Boddicker, R. L., Johnson, J. S., Weber, T.E., Ross, J.W., Baumgard, L.H., Gabler, N. K. (2013b). Heat stress reduces intestinal barrier integrity and favors intestinal glucose transport in growing pigs. *PLoS One, 8*(8), e70215. doi:10.1371/journal.pone.0070215

Phillips, N. A., Welc, S. S., Wallet, S. M., King, M. A., & Clanton, T. L. (2015). Protection of intestinal injury during heat stroke in mice by interleukin-6 pretreatment. *The Journal of Physiology, 593*(3), 739-753. doi:10.1113/jphysiol.2014.283416

Quiniou, N., Dubois, S., & Noblet, J. (2000). Voluntary feed intake and feeding behaviour of group-housed growing pigs are affected by ambient temperature and body weight. *Livestock Production Science, 63*(3), 245-253. doi:10.1016/S0301-6226(99)00135-9

Quiniou, N., & Noblet, J. (1999). Influence of high ambient temperatures on performance of multiparous lactating sows. *Journal of Animal Science, 77*(8), 2124. doi:10.2527/1999.7782124x

Quiniou, N., Noblet, J., van Milgen, J., & Dubois, S. (2001). Modelling heat production and energy balance in group-housed growing pigs exposed to low or high ambient temperatures. *British Journal of Nutrition, 85*(1), 97-106. doi:10.1079/BJN2000217

Renaudeau, D., Anais, C., Tel, L., & Gourdine, J. L. (2010). Effect of temperature on thermal acclimation in growing pigs estimated using a nonlinear function1. *Journal of Animal Science, 88*(11), 3715-3724. doi:10.2527/jas.2009-2169

Renaudeau, D., Gourdine, J. L., & St-Pierre, N. R. (2011). A meta-analysis of the effects of high ambient temperature on growth performance of growing-finishing pigs. *Journal of Animal Science, 89*(7), 2220-2230. doi:10.2527/jas.2010-3329

Sanz Fernandez, M. V., Johnson, J. S., Abuajamieh, M., Stoakes, S. K., Seibert, J. T., Cox, L., Kahl, S., Elsasser, T. H., Ross, J. W., Isom, S. C., Rhoads, R. P., Baumgard, L. H. (2015). Effects of heat stress on carbohydrate and lipid metabolism in growing pigs, *Physiol Rep,* 3 ( 2), 2015, e12315, doi: 10.14814/phy2.12315

Shi-bin, Y., Dai-wen, C., Ke-ying, Z., & Bing, Y. (2007). Effects of oxidative stress on growth performance, nutrient digestibilities and activities of antioxidative enzymes of weanling pigs. *Asian - Australasian Journal of Animal Sciences, 20*(10), 1600-1605. doi:10.5713/ajas.2007.1600

Silanikove, N. (2000). *Effects of heat stress on the welfare of extensively managed domestic ruminants* Elsevier B.V. doi:10.1016/S0301-6226(00)00162-7

Statista, 2017. Statista: The statistic Portal. https://www.statista.com/ accessed date Feb 2, 2019.

St-Pierre, N. R., Cobanov, B., & Schnitkey, G. (2003). Economic losses from heat stress by US livestock industries.*Journal of Dairy Science, 86*(13 Electronic Supplement 1), E77. doi:10.3168/jds.S0022-0302(03)74040-5

Surai, P. F. (2014). Polyphenol compounds in the chicken/animal diet: From the past to the future. *Journal of Animal Physiology and Animal Nutrition, 98*(1), 19-31. doi:10.1111/jpn.12070

Taïwe, G. S., & Kuete, V. (2017). In Kuete V. (Ed.), *Chapter 2 - management of inflammatory and nociceptive disorders in Africa.* Academic Press. doi://doi.org/10.1016/B978-0-12-809286-6.00002-9.

Tendergy, R. P. (1990). The role of vitamin E in immune response and disease resistance. *Annals of the New York Academy of Sciences, 587*(1), 24-33. doi:10.1111/j.1749-6632.1990.tb00130.x

van Heugten, E., Sweer, L.A., Stumpf, T.T., Risley, C.R. and Schell, T.C. (1997). Effects of water supplementation with selenium and vitamin E on growth performance and blood selenium

and serum vitamin E concentrations in weanling pigs. J. Am. Vet. Med. Assoc. 211:1039-1042.

Vergauwen, H., Tambuyzer, B., Jennes, K., Degroote, J., Wang, W., De Smet, S., Michiels, J., and van Ginneken, C. (2015). Trolox and ascorbic acid reduce direct and indirect oxidative stress in the IPEC-J2 cells, an in vitro model for the porcine gastrointestinal tract. *PLoS One, 10*(3), e0120485. doi:10.1371/journal.pone.0120485

Wang, Y. H., Leibholz, J., Bryden, W. L., & Fraser, D. R. (1996). Lipid peroxidation status as an index to evaluate the influence of dietary fats on vitamin E requirements of young pigs. *British Journal of Nutrition, 75*(1), 81-95. doi:10.1079/BJN19960112

Wei, A., & Shibamoto, T. (2007). Antioxidant activities and volatile constituents of various essential oils. *Journal of Agricultural and Food Chemistry, 55*(5), 1737-1742. doi:10.1021/jf062959x

Wilburn, E. E., Mahan, D. C., Hill, D. A., Shipp, T. E., & Yang, H. (2008). An evaluation of natural (RRR-α-tocopheryl acetate) and synthetic (all-rac-α-tocopheryl acetate) vitamin E fortification in the diet or drinking water of weanling pigs. *Journal of Animal Science, 86*(3), 584-591. doi:10.2527/jas.2007-0377

Windisch, W., Schedle, K., Plitzner, C., & Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine and poultry1. *Journal of Animal Science, 86*(suppl_14), E148. doi:10.2527/jas.2007-0459

Yang, C., Chowdhury, M. A. K., Huo, Y., & Gong, J. (2015). Phytogenic compounds as alternatives to in-feed antibiotics: Potentials and challenges in application. *Pathogens (Basel, Switzerland), 4*(1), 137-156. doi:10.3390/pathogens4010137

Zhang, H., & Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects.*Current Opinion in Food Science, 8,* 33-42. doi:10.1016/j.cofs.2016.02.002

Zhang, T., Zhou, Y. F., Zou, Y., Hu, X. M., Zheng, L. F., Wei, H. K., Giannenas, I., Jin, L.Z., Peng, J. & Jiang, S. W. (2015). Effects of dietary oregano essential oil supplementation on the stress response, antioxidative capacity, and HSPs mRNA expression of transported pigs. *Livestock Science, 180,* 143-149. doi:10.1016/j.livsci.2015.05.037

Zhu, L. H., Zhao, K. L., Chen, X. L., & Xu, J. X. (2012). Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs1. *Journal of Animal Science, 90*(8), 2581-2589. doi:10.2527/jas.2011-4444

Zou, Y., Hu, X. M., Zhang, T., Wei, H. K., Zhou, Y. F., Zhou, Z. X., & Peng, J. (2016). Effects of dietary oregano essential oil and vitamin E supplementation on meat quality, stress response and intestinal morphology in pigs following transport stress. *The Journal of veterinary medical science, 79*(2), 328–335. doi:10.1292/jvms.16-0576

# CHAPTER II:

## Supplementation of antioxidants to improve production efficiency and health of growing pigs housed under heat stressed conditions

**ABSTRACT:** The objective of this study was to evaluate the impact of vitamin E or polyphenols supplemented in feed or drinking water as a heat abatement strategy in growing pigs. The study consisted of 2 replicated experiments. In each experiment, 64 crossbred pigs (32 gilts and 32 barrows) were used, with an initial body weight of 50.7±3.8 kg and 43.9± 3.6 and age of 13-weeks and 12-weeks old for each experiment, respectively. Individually housed pigs were assigned within weight blocks and sex to a 2 x 4 factorial arrangement consisting of 2 environments (thermo-neutral (21.2°C) or heat-stressed (30.9°C)) and 4 supplementation treatments (control diet (25 IU/kg dl-α-tocopheryl acetate); control+100 IU/L d-α-tocopherol in water; control+200 IU/kg of dl-α-tocopheryl acetate in feed; or control+400 mg/kg of polyphenols in feed). Supplementation was started 7 d prior to temperature treatments applied for 28 d. Heat stress reduced ($P \leq 0.001$) final BW, ADG, and ADFI (-7.4 kg, -26.7%, and -25.4%, respectively) and increased ($P < 0.001$) respiration rate and rectal temperature, but no effects of supplementation were detected. Intestinal morphology in the jejunum and ileum was not affected by heat stress or supplementation treatments or their interaction ($P \geq 0.05$). Cellular proliferation was reduced in the jejunum but not in the ileum ($P = 0.037$) and was increased when vitamin E was supplemented in feed or in water under the heat stress environment (interaction, $P = 0.04$). Serum vitamin E concentrations increased ($P < 0.001$) with vitamin E supplementation for control, vitamin E in water and vitamin E in feed (1.64 vs. 3.59 and 1.64 vs. 3.24) but not for polyphenol (1.64 vs. 1.67 mg/kg), and was greater when supplemented in water vs. feed ($P = 0.002$), and when measured on d 28 vs. d 2 under the thermo-neutral environment (interaction, $P = 0.016$) but not the heat-stressed environment. Liver vitamin E increased ($P < 0.001$) with vitamin E supplementation, especially when supplemented in water, but not polyphenols (3.9, 31.8, 18.0, 4.9 ppm for control, vitamin E in water, vitamin E in feed, and polyphenols, respectively). Serum malondialdehyde (MDA) was

greater ($P < 0.05$) for antioxidant supplementation compared to control pigs, and heat stress reduced serum MDA on d 2, but increased on d 28 (interaction, $P < 0.001$). MDA in ileum was increased by heat stress when using vitamin E or polyphenols in feed (interaction, $P = 0.005$). Cytokines in serum were not impacted by heat stress or antioxidant supplementation ($P \geq 0.05$), but TNF-α in jejunum and ileum mucosa were decreased by heat stress ($P < 0.05$). TNF-α in ileum was reduced by vitamin E supplementations under heat stress (interaction, $P < 0.001$). Red blood cells, hemoglobin and hematocrit percentage were reduced by heat stress on d 28 (interaction, $P < 0.05$), and white blood cell, platelets, neutrophils, and monocytes counts decreased on d 28 compared to d 2 ($P < 0.001$). The addition of the antioxidants in feed or in drinking water did not alleviate the negative impact of heat stress in growing pigs.

**Key words:** growing pigs, heat stress, immune status, oxidative status, polyphenols, vitamin E

## Introduction

High environmental temperatures negatively affect pig production performance, causing significant economic losses (St-Pierre *et al*., 2003). During high-temperature conditions, pigs struggle to regulate core temperature because of their dysfunctional sweat glands (Quiniou *et al*, 2000). This condition causes pigs to be more susceptible to heat stress, which negatively affects growth performance (White *et al*., 2008), reduces feed intake (Quiniou *et al*., 2000), increases respiration rate and rectal temperature (Quiniou and Noblet, 1999; Patience *et al*., 2005), reduces intestinal barrier integrity (Oliver *et al*., 2012; Pearce *et al*., 2013), reduces meat quality (Montilla *et al*., 2014; Volodina *et al*., 2017), alters metabolism (Sanz Fernandez *et al*., 2015), and causes oxidative stress (Sakaguchi *et al*., 1995; Volodina *et al*., 2017).

Oxidative stress is defined as an imbalance between oxidants and the natural antioxidant system in the body (Zhang and Tsao, 2016). Oxidative stress is produced by reactive oxygen

species (ROS) such as hydroxyl radical (HO·), peroxyl radical (ROO·), hydrogen peroxide ($H_2O_2$), superoxide anion radical ($O_2$·) and singlet oxygen ($^1O_2$) (Sies *et al*., 1992). These molecules produce oxidation in the cells, causing damage to DNA, proteins, and lipids, altering the normal functioning of the cell, and producing measurable byproducts, including 8-hydroxydeoxyguanosine (8-OHdG), protein carbonyls and malondialdehyde (MDA), respectively (Reuter *et al*., 2010).

The first line of defense against oxidants is the endogenous antioxidant system, which includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Pisoschi and Pop, 2015). The second line of defense is antioxidants provided in the diet, such as vitamin E, vitamin C, carotenoids, polyphenols, selenium, and zinc (Pisoschi and Pop, 2015).

Vitamin E is a fat-soluble vitamin and serves as a natural antioxidant in the body. d-α-Tocopherol is the most biologically active form of vitamin E (Sies, 1992) and dl-α-tocopheryl acetate is the most common source of vitamin E used for animal diets. Vitamin E prevents lipid peroxidation by scavenging ROS and donating electrons abstracted by free radicals from biomolecules (Sies *et al*., 1992). Supplementation of vitamin E together with selenium improved intestinal epithelial barriers and alleviated oxidative stress in growing pigs housed under heat stress (Liu *et al*., 2016). Vitamin E increased immune responses in broilers (Niu *et al*., 2009), egg production in laying hens (Bollengier-Lee *et al*., 1999) and feed intake in hens housed under heat stress (Kirunda *et al*., 2001). On the other hand, Niu *et al*. (2009) reported no effects of vitamin E supplementation on BW, ADFI, and G:F in broilers housed under heat stress conditions. Natural vitamin E (d-α-tocopherol) supplementation in drinking water of pigs showed high absorption of vitamin E (van Heugten *et al*., 1997 and Wilburn *et al*., 2008), and may be strategically used to decrease negative effects of heat stress in pigs.

Polyphenols are found in plants and serve to protect against insects, ultraviolet light and physical damage (Surai, 2014). Polyphenols have antioxidant properties preventing damage by ROS, can activate antioxidant enzymes and inhibit oxidases. Supplementation of polyphenols in the diet decreased MDA concentrations in plasma (Zhang *et al*., 2014) and reduced diarrhea and E. *coli* excretion in weaned piglets (Verhelst *et al*., 2014). In humans, polyphenols caused a reduction in DNA damage in lymphocytes (Smith *et al*., 1999).

We hypothesized that supplementation of vitamin E and polyphenols in feed or drinking water could enhance growth performance, intestinal health, oxidative and immune status in growing pigs housed under heat stress conditions.

**Materials and methods**

The experimental protocol and procedures for the present study were reviewed and approved by the Institutional Animal Care and Use Committee of North Carolina State University (Raleigh, NC, US).

***Animals and experimental design***

Two replicate experiments were conducted. In each experiment, 64 crossbred pigs (32 gilts and 32 barrows) with an initial body weight of 50.7 ± 3.8 and 43.9 ± 3.6 kg and age of 13 and 12 weeks for experiment 1 and 2, respectively, were used. Pigs were blocked by initial body weight and sex and randomly assigned within blocks to 1 of 8 treatments using an experimental allotment program (Kim and Lindemann, 2007). Treatments consisted of 2 environmental treatments (thermo-neutral and heat-stressed) and within each environment, 4 dietary treatments. There was a combined total of 128 individually housed pigs represented in this study, with 16 pigs per experimental treatment.

Within each experiment, 2 rooms located at the Swine Research Complex (Raleigh, NC) were used. The capacity of each room was 32 pigs and pigs were housed individually in each pen (dimensions of each pen were 0.91 x 1.82 m). Each pen contained a cup waterer (Aquachief, Hog Slat, Newton Grove, NC) in the back of the pen and an individual stainless-steel feeder (Boar feeder, Hog Slat) in the front of each pen. All pigs were provided *ad libitum* access to feed and drinking water.

Immediately after pigs were allocated, they were provided water supplementation or dietary treatments for 7 days prior to the initiation of temperature treatments (adaptation period). Both rooms were set at a constant temperature of 22°C during these 7 days and after this period was completed, the 2 environmental treatments (thermo-neutral and heat-stressed) were implemented for the subsequent 28 days. The heat-stressed and thermo-neutral environmental treatments were represented by 1 room each within each experiment, resulting in 2 replicate rooms per environmental treatment for the study. Each room was equipped with an environmental control system (GL-5124LW Grower Direct, Monitrol Inc., Boucherville, Quebec, Canada) to mimic high temperatures as commonly experienced in the summer season and normal thermo-neutral conditions, respectively. Temperatures for the heat-stressed room were set at 28.3, 29.4, 29.4, 31.1, 32.8, 33.3, 34.4, 35.6, 34.4, 31.7, 29.4 and 29.4°C for 2400, 0200, 0400, 0600, 0800, 1000, 1200, 1400, 1600, 1800, 2000, and 2200h, respectively. For the thermo-neutral room, temperatures were set at 18.9, 18.9, 20.0, 20.0, 21.1, 21.1, 22.2, 21.1, 21.1, 20.0, and 20.0°C for 2400, 0200, 0400, 0600 0800, 1000, 1200, 1400, 1600, 1800, 2000, and 2200h, respectively. Temperatures were recorded every 10 min using 3 data loggers (Logtag, Micro DAQ Ltd., Contoocook, NH) located throughout each room at the near, middle and far end of the door and approximately the same

height as the pigs. Photoperiod was fixed at 12 h of artificial light from 0700 to 1900 h and a ventilation rate of 100 cfm.

The supplementation treatments were applied as follows: 1) control diet (25 IU/kg dl-α-tocopheryl acetate) (CON); 2) control diet + 100 IU/L d-α-tocopherol in water (VEW) (Emcelle® tocopherol, Stuart products, Bedford, TX); 3) control diet + 200 IU/kg of dl-α-tocopheryl acetate in feed (VEF) (Rovimix®, DSM, Heerlen, The Netherlands); and 4) control diet + 400 mg/kg of a commercial blend of plant-based polyphenols (POL). The dietary treatment feeds were manufactured at the North Carolina State University Feed Mill Educational Unit (Raleigh, NC). Diets were based on corn-soybean meal and were formulated to contain 2.78 g standardized ileal digestible lysine per Mcal ME (Table 1) and met or exceeded all nutrient requirements, including vitamin E, for growing pigs as suggested by the NRC (2012). A basal mix containing all ingredients, except the test ingredients, was first created and divided into 4 batches. No supplements were added to the control treatment and the treatment receiving vitamin E in the water. Vitamin E or plant polyphenols were mixed with the basal diet to create the vitamin E and plant polyphenol diets, respectively.

To prepare the water supplementation treatment, a stock solution was prepared by adding concentrated vitamin E to water at a ratio of 0.0256:1. The vitamin E stock solution was subsequently metered into the drinking water at a rate of 1:128 vitamin E stock solution:drinking water using a water medication device (Dosatron DM11F, Hog Slat, Newton Grove, NC). Treated water was supplied to randomly selected pens (within block) within each room (8 pens per room) using a separate water line connected to these pre-selected pens. To determine water disappearance both rooms were equipped with 4 water meters each. Two water meters (Elster C700 digital Invision 5/8” X 3/4” bronze valve, Elster AMCO Water, Ocala, FL ) were located in the principal

water system (1 on each side of the room) to measure water consumption for treatments that were not receiving water supplementation (24 pens in each room), and 2 water meters (water meter 5/8" Arad, AradGroup, Dalia, Israel) were used to measure water intake for the water supplementation treatment (8 pens in each room).

***Growth performance and water intake***

Body weight (BW) was measured on d -7 (7 d prior to the initiation of heat stress), and d 0, 7, 14, and 28 to calculate average daily gain (ADG). Daily feed intake was measured from the difference between daily feed additions and feed remaining at the end of each weekly period divided by 7 days. Feed efficiency (G:F) was calculated by dividing ADG by ADFI. Water intake was determined weekly by subtracting the reading on each water meter at the beginning of the period from the reading at the end of the period.

***Respiration rate and rectal temperature***

Respiration rate and rectal temperature were measured at d 0 (prior to the initiation of the environmental treatments) as a baseline before heat stress was initiated. Likewise, respiration rate and rectal temperature were measured on d 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 of heat stress between 1300 and 1600 h (peak of heat stress during the day). Respiration rate was determined by counting the number of flank movements during a 30 s period at rest, using a stopwatch by the same observer during the entire experiment. Rectal temperature was measured using a digital thermometer (GLA M700 GLA Agricultural Electronics, San Luis Obispo, CA) after respiration measurements were completed.

***Sample collection***

Blood samples from each pig were collected by venipuncture (jugular vein) using 20-gauge x 3.8 cm multiple use drawing needles (Vacuette, Greiner bio-one, Kremsmunster, Austria) on d 2 and d 28 (at 1200). Blood for serum analysis was collected into 10 mL vacuum tubes (BD Vacutainer Serum, Franklin Lakes, NJ). Blood was centrifuged at 4,000 x g for 10 min at 4°C using a refrigerated centrifuge (Centra GP8R, Thermo IEC, Waltham, MA) and serum supernatant was collected. Serum was aliquoted into 3 tubes of 2 mL capacity (Biotix, Neptune, 3472.X, Mesa Rim, San Diego, CA) and stored at -80°C for further analysis. Blood for complete blood count (CBC) analysis was collected into 6 mL vacuum tubes (BD Vacutainer containing 10.8 mg $K_2$EDTA) and immediately submitted to Antech Diagnostic Laboratory (Cary, NC) for analysis.

At the end of each experiment (d 28), 16 pigs in each room (64 pigs total; 8 pigs per experimental treatment) were euthanized using a captive bolt gun, followed by exsanguination. Blood samples were collected after exsanguination of euthanized pigs and the blood samples were processed as indicated previously and stored at -80°. The abdominal cavity was opened and 25 cm of the proximal jejunum (anterior to the duodenal-jejunal junction) and 25 cm of distal ileum (10 cm proximal to the ileal-cecal junction) were excised. Mucosa samples from the proximal jejunum and distal ileum were scraped using a glass slide, placed into 2 mL tubes (Biotix, Neptune, San Diego, CA), snap frozen in liquid nitrogen at -80°C, and subsequently stored in a -80°C freezer until further analysis was conducted. Four cm of intact intestinal tissue from the jejunum and ileum was collected, rinsed in 0.9% saline, and fixed in 40 mL of 10% formaldehyde solution for 3 d for further histological measurements. Approximately 100 g of liver from the center of the right lobule was collected and stored in a plastic bag at -20°C for vitamin E analysis.

***Chemical Analyses***

Proximate analysis of the diets was conducted by the Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO) using AOAC Official methods (2006). Diets were analyzed for moisture (Method 934.01), crude protein (Method 990.03), crude fat (Method 920.39 (A)), crude fiber (Method 978.10), ash (Method 942.05), neutral detergent fiber (JAOAC 56, 1352-1356, 1973), acid detergent fiber (Method 973.18 (A-D)), calcium (Method 985.01 (A, B, D)) and phosphorus (Method 966.01).

Concentrations of vitamin E (IU/kg) in feed samples and in drinking water (IU/ml) samples were analyzed by DSM Technical Marketing Analytical Services (Parsippany, NJ, US), using a high performance liquid chromatography system with fluorescence detection following AOAC Official Method 971.30 (2006) for α-tocopherol and α-tocopheryl acetate determination in foods and feeds.

The vitamin E concentrations in serum samples collected on d 2 and 28 and concentrations of vitamin E in the liver were determined by the Veterinary Diagnostic Laboratory at Iowa State University (Ames, IA) using high performance liquid chromatography.

***Intestinal measurements***

Samples collected from the jejunum and ileum were submerged for 3 days in 10% formalin at room temperature. After this period, cross sections of 0.4 cm thick of each section were taken and 2 to 3 sections per pig were stored into cassettes submerged in 10% formalin by the North Carolina State University College of Veterinary Medicine Histopathology Laboratory (Raleigh, NC) for hematoxylin and eosin (H&E) and for Ki-67 staining of slides. Each microscope slide was photographed using an AmScope®FMA050 microscope (AmScope®, Irvine, CA) and AmScope®3.7 software (AmScope®, Irvine, CA) to capture and analyze images at 40x

magnification. Eighteen randomly positioned villi and crypts were selected to measure villus height (from top of the villus to the crypt junction), villus width (from the middle of the length of the villus), and crypt depth (from the crypt junction to the base of the crypt) based on the methods described by Touchette *et al*. (2002). Villus height and crypt depth ratio was obtained by dividing the villus height by its own crypt depth.

The proliferation rate of cells in the crypts was measured by staining for protein Ki-67. Microscope slides were scanned using 100x magnification using an AmScope® FMA050 microscope and AmScope software version 3.7. Images of fifteen crypts per sample were captured and evaluated using the Image JS software (Almeida *et al*., 2012). The ratio of Ki-67 positive cells in each crypt of the jejunum and ileum tissue was calculated by dividing Ki-67 positive cells by total cells in the crypt. Ki-67 is a protein located in the nucleus of proliferating cells and stained with a Ki-67 antibody. The same person recorded all morphological analysis.

***Concentration of cytokines in mucosa and serum***

Tumor necrosis factor-α (TNF-α) was measured in the mucosa of the proximal jejunum and distal ileum. Samples (0.75 to 0.80 g of mucosa) were combined with 1.5 mL of phosphate buffered saline (PBS; pH = 7.4) and subsequently homogenized using a Tissuemiser (Pro 200, Pro Scientific Inc., Willenbrock, Oxford, CT). The samples were then centrifuged at 15,000 x g at 4°C for 20 min. A 1 mL sample of supernatant was obtained and stored at -80°C until it was analyzed. Total protein was evaluated in mucosal samples prior to TNF-α analysis using the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL). A porcine TNF-α ELISA kit (Quantitine® R&D Systems, Inc. Minneapolis, MN) was used to analyze TNF-α. Mucosal concentrations of TNF-α were expressed in pg/mg of total protein.

Serum samples collected on d 2 and 28 were submitted to Eve Technologies Corporation (Calgary, Canada) for analysis of pro- and anti-inflammatory cytokines using the Luminex xMAP Multi-plex technique (multiplex technology). Cytokines analyzed included interferon (IFN)-$\gamma$, interleukin (IL)-1$\alpha$, IL-1$\beta$, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and TNF-$\alpha$ and were expressed in pg/mL of serum.

***Oxidative status in mucosa and serum***

Malondialdehyde (MDA) analysis was conducted in the mucosa of the ileum and jejunum. Samples of mucosa (100 mg) were homogenized using a Tissuemiser (Pro 200, Pro Scientific Inc., Willenbrock, Oxford, CT) using 1 mL of PBS and 10 µL of butylated hydroxytoluene (BHT). Concentrations of MDA in mucosal tissues and serum samples were analyzed using the Oxiselect™ TBARS Assay Kit Protocol (MDA Quantitation; Cell BioLabs, Inc., San Diego California). Only ileum results were measured because jejunum samples were compromised during analysis at the last step of the assay. Absorbance was measured at 532 nm in a multi-detection micro-plate reader (Synergy HT, BioTek Instruments, Winooski, VT). Results from MDA for ileum mucosa and serum samples were expressed in µM/g of total protein and µM, respectively.

***Statistical analysis***

Data were analyzed using the Proc MIXED procedure of SAS (v.9.4, SAS Institute. Inc., Cary, NC). Individual pig was used as the experimental unit. The model included environmental treatment, antioxidant supplementation treatments, and their interaction. Block nested within environment was used as the random effect. The LSD method was used to determine differences between means following a significant F-test. Statistical significances were considered at $P < 0.05$ and tendencies at $0.05 \leq P \leq 0.10$.

## Results

### *Room temperature, relative humidity, and water consumption*

The mean temperatures for the thermo-neutral room and heat stress rooms were 20.5 ± 1.66°C and 30.0 ± 3.46°C, respectively for Exp. 1 and 21.8 ± 3.66°C and 31.7 ± 3.1°C, respectively for Exp. 2. Room temperatures fluctuated within day, which was consistent with the experimental design (Figure 1A and 1B). The relative humidity for the thermo-neutral room and heat stress room was 54.6 and 52.4 %, respectively Exp. 1, and 65.4 and 47.4 %, respectively for Exp. 2.

Water disappearance per pig for the thermo-neutral room and heat stress room was 11.3 and 6.7 liters, respectively ($P = 0.061$). The daily water disappearance per pig provided vitamin E supplementation in water in the heat-stressed room was lower than that of pigs given vitamin E in water in the thermo-neutral room (6.8 vs. 14.3 liters). The average of water disappearance of pigs for control, dietary vitamin E and polyphenols treatments was 7.5 liters per day and for pigs supplemented with vitamin E in water it was 10.6 liters per day ($P = 0.067$).

### *Growth performance*

In Exp. 1, 1 pig (thermo-neutral and polyphenol treatment) was removed from analysis due to poor growth. In Exp, 2, 2 pigs (thermoneutral and dietary polyphenol and vitamin E treatments) were removed due to excessive weight loss related to suspected ileitis (*Lawsonia intracellularis*). Subsequently, all pigs were individually treated daily from d 9 to d 21 of the experiment with an oral dose of 8.8 mg/kg BW·$d^{-1}$ of tiamulin hydrogen fumarate (Denagard 12.5%-Elanco Animal Health, Greenfield, IN). Three pigs (2 from thermo-neutral and vitamin E in water treatment, and 1 pig from heat-stress and control treatment), were medicated until the end of the study and 1 pig died (heat-stressed environment and dietary vitamin E treatment).

BW decreased ($P < 0.05$) in heat stressed pigs during the last two weeks of the experiment but not during the first two weeks (Table 2). ADG and ADFI were reduced by the heat stressed environment ($P < 0.05$) from d 0 to 7, d 7 to 14, d 14 to 21, d 21 to 28 ($P < 0.05$) and overall ($P < 0.001$). Pigs were negatively affected by heat stress, decreasing G:F from d 0 to 7 ($P = 0.03$) and for the overall period ($P = 0.002$). Dietary and water supplementation treatments did not significantly impact ADG, ADFI, or G:F either in heat stressed or thermo-neutral pigs. In the first and second week of the experiment, ADG was decreased by supplementation of antioxidants for the heat stress environment, but not the thermo-neutral environment (interaction, $P = 0.051$ and $P = 0.085$, respectively). Additionally, in the first and the second week, supplementation treatments reduced G:F in the heat stress environment only (interaction, $P = 0.076$ and $P = 0.048$, respectively).

***Respiration rate and rectal temperature***

Respiration rate and rectal temperature was measured immediately prior to the initiation of heat stress and they were not different due to environment or supplementation treatments ($P \geq 0.05$). The heat-stressed environment increased respiration rate ($P < 0.001$; Figure 2A) and rectal temperatures in pigs (Figure 2B; $P < 0.001$). No significant differences in respiration rate or rectal temperature were detected among supplementation treatments ($P \geq 0.05$). Respiration rate and rectal temperature decreased over the course of the experiment for both the thermo-neutral and heat-stressed environments.

***Histology and immunohistochemistry in the gut***

Villus height, villus width and crypt depth in the jejunum and ileum were not affected by environment, supplementation treatments, or their interaction ($P > 0.05$; Table 3). Villus/crypt ratio in the jejunum increased by dietary vitamin E supplementation treatments compared with

control ($P$ = 0.046; +17.6%). Cellular proliferation measured with ki-67 staining was reduced by heat stress in the jejunum (-12.8%), but not the ileum ($P$ = 0.037). Moreover, proliferation of enterocytes was increased by dietary vitamin E and vitamin E in drinking water treatments in the heat-stressed environment, but not the thermo-neutral environment (interaction, $P$ = 0.04).

***Concentration of vitamin E in serum and liver***

The concentration of vitamin E in serum was increased by supplementation ($P$ < 0.001) of vitamin E in water and in feed when compared with control and polyphenol treatments (3.59, 3.24, 1.64 and 1.67 ppm, respectively; (Figure 3). Serum vitamin E concentration was greater when measured on d 28 vs. d 2 (2.62 vs 2.45; $P$ = 0.067). Vitamin E in serum was increased by supplementation of vitamin E in feed and in drinking water when compared with control and polyphenols treatment on d 28 in the thermo-neutral environment (interaction, $P$ = 0.016) but not in the heat-stressed environment (Table 4).

Supplementation of vitamin E in feed and in water increased vitamin E concentration in liver tissue ($P$ < 0.001; Table 4). The addition of vitamin E in water increased vitamin E in the liver to a greater extent when compared with dietary vitamin E supplementation ($P$ < 0.05). Dietary polyphenol treatment did not affect vitamin E concentration in the liver ($P$ < 0.05). No significant differences ($P \geq 0.05$) in liver vitamin E concentrations were found due to thermal environment or the interaction of environment and supplementation.

***Cytokines and oxidative status on mucosa and serum***

MDA in serum was increased by dietary vitamin E, vitamin E in water and dietary polyphenol treatments ($P$ = 0.017), but not by environment or the interaction between environment and supplementation ($P \geq 0.05$; Table 5). Serum concentrations of MDA were increased when measured on d 28 ($P$ < 0.001) compared with MDA levels on d 2. Moreover, MDA was reduced

by heat stress on d 2, but increased on d 28 by the heat-stressed environment (interaction, $P < 0.001$). Additionally, heat stress and antioxidant supplementations did not significantly impact MDA concentration in ileum mucosa ($P \geq 0.05$). However, MDA concentrations were increased by heat stress when vitamin E and polyphenols were supplemented, but not when vitamin E was provided in the water (interaction, $P = 0.005$).

Serum concentrations of IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, and TNF-α were not impacted by environment, supplementation or their interaction ($P \geq 0.05$; Table 6). Serum concentrations of IL-8 were reduced ($P < 0.05$) and IL-1ra concentration tended to be increased ($P = 0.056$) by heat stress, but no effects due to supplementation or interactions were observed ($P \geq 0.05$). IFN-γ and IL-8 were higher on d 28 ($P = 0.078$ and $P = <0.001$) compared to d 2. In contrast, IL-1ra, IL-12 and IL-18 were reduced ($P < 0.05$) on d 28 compared to d 2.

The concentration of TNF-α in mucosa of the jejunum was decreased ($P = 0.022$) by the heat-stressed environment, and the supplementation of vitamin E in water increased TNF-α in jejunum ($P = 0.064$; Table 6). TNF-α concentration in the mucosa of the ileum was decreased by the heat-stressed environment ($P < 0.05$) and vitamin E in water, but not dietary vitamin E or polyphenol treatments ($P < 0.001$). TNF-α was reduced in the ileum mucosa by vitamin E supplementation, but not dietary polyphenols in the heat-stressed environment (interaction, $P < 0.001$).

***Complete blood count (CBC)***

Red blood cells, hemoglobin, and hematocrit percentage were reduced on d 28 by the heat-stressed environment, but this was not the case on d 2 (interaction, $P < 0.05$; Table 7). White blood cells, platelets, neutrophils, and monocytes counts decreased ($P < 0.001$) on d 28 compared to d 2, but no other differences were observed.

**Discussion**

Heat stress reduces growth performance in pigs as shown by St-Pierre *et al*. (2003) and Pearce *et al*. (2014). In the present study, BW was not affected by heat stress on d 7, but it was reduced on d 14, 21 and 28. These responses were in agreement with those reported by Quiniou *et al*., (2000) and White *et al*., (2008), but not with those reported by Mendoza *et al*. (2017, who did not report statistical differences in BW on d 14 and 28 in growing pigs (39 kg of initial BW). The impact of heat stress on performance of pigs is dependent on pig BW as shown by Quiniou *et al*. (2000), who observed a greater negative impact on BW due to high temperatures in heavier pigs (90 kg of BW) in comparison with lighter pigs (30 kg of BW). Reductions in ADFI are closely associated with reductions in BW and ADG in growing pigs (Renaudeau *et al*., 2011). The reduction in ADFI can be more severe during stress factors such as heat stress. During high temperature environments, the body reacts by decreasing or avoiding any other extra heat production that could increase core body temperature, including high feed intake. In the present study, ADG and ADFI in growing pigs were negatively impacted by heat stress throughout the experiment, which is in agreement with the results reported by Collin *et al*. (2001), Kirunda *et al*. (2001), Kerr *et al*. (2003), Pearce *et al*. (2014), and Mendoza *et al*. (2017). Likewise, in the present study, the heat-stressed environment negatively affected G:F, contradicting results reported by Mendoza *et al*. (2017) in growing pigs, who reported increased G:F.

Clearly heat stress reduced performance in the present study and we hypothesized that the use of antioxidants could ameliorates the negative effects of heat stress in growing pigs. The supplementation of vitamin E in water and dietary vitamin E and polyphenols did not affect BW, ADFI, or G:F, regardless of environmental temperature. Niu *et al*. (2009) reported that the addition of vitamin E in the diet did not affect BW or ADFI, but G:F was decreased using 100 ppm of

dietary vitamin E in broilers and no effects were observed using 200 ppm of vitamin E. Hasty *et al*. (2002) reported a reduction in feed efficiency when vitamin E was supplemented in the diet, but no statistical differences were detected for ADG and ADFI in growing pigs. Dietary polyphenols (grape pomace) included at 7.5% (Ebrahimzadeh *et al*., 2018) and 0.1% of a blended polyphenol additive (Zhang *et al*., 2014) did not show any significant difference on growth performance when used in broilers and weaned piglets, respectively. In the present study, polyphenols did not affect growth performance of pigs. The response to dietary polyphenols can be affected by differences in absorption, metabolism, and interaction with other nutrients (Surai *et al*., 2014).

The use of water by pigs to drink and spray themselves to reduce core body temperature is expected to be higher under high temperature environment. In the present study, the estimated consumption of drinking water for pigs housed under the heat-stressed environment was 40.7% lower than the thermo-neutral environment. This is similar to Vajrabukka *et al*. (1981), who showed that fattening pigs consumed less water at high environmental temperatures (24 to 35 ºC). Patience *et al*. (2005) reported less water consumption during the hot periods of the day in 25-kg BW pigs. Pigs in the present study only had access to cup waterers with a nipple inside to minimize water wastage associated with behavioral changes such as wetting of the skin to increase evaporative heat losses. The water in the heat stress rooms was warm due to the high temperature of the rooms and may have caused the lower water consumption of pigs compared to the pigs housed in the thermo-neutral room. In addition, pigs given water with supplemental vitamin E in the heat stressed room consumed 52.4% less water than their peers in the thermo-neutral room (6.8 vs. 14.3 L/d), whereas water intake was similar for pigs given control water (6.7 and 8.2 L/d for

the heat stress and thermo-neutral rooms, respectively). Perhaps the high temperature in the heat-stressed room altered the taste of vitamin E, reducing water consumption by the pigs.

High respiration rate and rectal temperature are positively correlated with heat stress in pigs when temperatures exceed 25ºC temperature (Yu *et al*., 2010, Liu *et al*., 2016, Mendoza *et al*., 2017 and Cervantes *et al*., 2018). High body temperature is associated with thermoregulatory mechanisms sending blood flow to the periphery to dissipate the excess heat (Kurz, 2008). This will decrease blood flow to visceral organs, causing damage to the small intestine due to hypoxia (Yu *et al*., 2010). In the present study, heat stress clearly increased respiration rate and rectal temperature throughout the study, but antioxidant supplementation did not ameliorate these effects. In contrast, Liu *et al*. (2016) showed that 200 IU/kg of dietary vitamin E and 1.0 ppm of selenium reduced oxidative stress caused by heat stress on intestinal barrier integrity *in vitro*. In the present study some acclimation to the heat-stress and thermo-neutral conditions was observed as indicated by a reduction in rectal temperature and respiration rate over time, similar to Renaudeau *et al*. (2010) and Cervantes *et al*. (2018).

Heat stress causes damage in the intestine due to reduced blood flow with heat stress. Yu *et al*. (2010) found damage to the tips of jejunal villi and shortening of villus height and decreased crypt depth with heat stress in pigs. Oliver *et al*. (2012) reported injury in the duodenal epithelium in mice exposed to heat stress. Similarly, Pearce *et al*. (2014) reported decreased villus height and villus:crypt ratio in the ileum and jejunum tissue of growing pigs exposed to heat stress. Contrarily to these results, in the present study no effects of environmental treatments on histology in ileum or jejunum (villi height, width, and crypt depth) were detected. However, heat stress increased cell proliferation in the jejunum as measured by Ki-67 staining.

The addition of dietary vitamin E increased the villus:crypt ratio in the jejunum. The addition of polyphenols in feed did not alter intestinal histology. Gessner *et al*. (2013) showed significant increases in villus height:crypt depth ratio in the duodenum of 6-week old piglets when using polyphenols (10 g/kg of grape seed and grape marc extract) in the diet. The addition of vitamin E in feed and in water improved cell proliferation in the ileum of pigs housed under heat stress condition, but not in pigs housed under thermo-neutral conditions, suggesting that the body accelerated the cellular proliferation to compensate for cellular death by hypoxia during heat stress when vitamin E, but not by polyphenols, was supplemented.

Several authors reported that heat stress reduced serum vitamin E concentration (Chow, 1991 and Bollengier-Lee *et al*., 1999) because vitamin E reacts against oxidation caused by heat stress, reducing its concentration in serum. In the current study, serum concentration of vitamin E was not impacted by heat stress, suggesting that heat stress in the pigs may not have been sufficiently severe to have significantly influenced serum vitamin E concentration. Serum vitamin E levels were higher when vitamin E was supplemented in the drinking water than dietary vitamin E. This is in agreement with Wilburn *et al*. (2008) who reported greater plasma vitamin E when vitamin E was added to water compared to dietary vitamin E. The increase in serum vitamin E concentration when supplemented in the water may be due to the natural vitamin E source which is absorbed faster than the synthetic vitamin E supplemented in feed (Wilburn *et al*., 2008). In the present study, serum vitamin E was increased +9.5% on d 28 when compared to d 2, showing that medium-term supplementation of vitamin E can increase serum vitamin E concentration over time. However, Wilburn *et al*. (2008) when using a natural dietary source of vitamin E in drinking water observed a reduction in plasma vitamin E on d 21 when compared with d 3 in weaned piglets. Serum vitamin E decreased on d 28 during heat stress environment compared to d 2, which is

supported by Chow (1991). Likewise, the efficiency of the absorption of natural vitamin E vs synthetic vitamin E is demonstrated in the current study by the fact that supplementation of vitamin E in feed and in water increased serum vitamin E concentrations by 10 and 22%, respectively, on d 28, but this was not the case for the polyphenol treatment (-6 %).

The addition of dietary polyphenols did not increase serum vitamin E concentrations in growing pigs on d 28, suggesting that polyphenols did not spare or regenerate vitamin E (Augustin *et al*., 2008). On the other hand, Luehring *et al.* (2011) showed that polyphenols in combination with low dietary vitamin E (7 $mg \cdot kg^{-1}$ of feed) increased vitamin E in plasma and in liver of growing pigs when using fish oil to induce oxidative stress.

Supplementation of vitamin E in the diet was associated with increased amounts of vitamin E in serum and liver tissue. The liver is the principle place of storage for vitamin E in the body. Thus, the supplementation of vitamin E in the feed and especially when provided via the water increased liver concentrations of vitamin E, but this was not the case when polyphenols were supplemented. The high concentrations of vitamin E in serum and liver with vitamin E supplementation in water could be due to the fact that the natural form of vitamin E (d-α-tocopherol) that was used is more bioavailable than the synthetic form (dl-α-tocopheryl acetate) that was used in the feed. Similarly, Wilburn *et al*. (2008) reported greater concentrations of vitamin E in serum and liver when using natural RRR-α-tocopheryl acetate in water compared to the synthetic all-*rac*-α-tocopheryl acetate. In addition, total intake of vitamin E per day, using the estimated water consumption for pigs supplemented with vitamin E in the water was 900 IU compared to 500 IU total vitamin E intake when supplemented in feed. Thus, part of the response is likely related to greater vitamin E intake when it was supplemented in the water.

MDA is a product produced during lipid peroxidation in the cell under oxidative stress (Grotto *et al*., 2009). In a study conducted by Montilla *et al*. (2014), MDA was 2.5-fold greater in 35-kg BW pigs during a short period of heat stress (1 d) compared with a thermo-neutral environment. Intestinal cells pre-treated *in vitro* with Trolox (a water-soluble form of vitamin E) showed markedly reduced oxidative stress when compared with intestinal cells pre-treated *in vitro* with ascorbic acid (Vergauwen *et al*., 2015). The inclusion of other dietary antioxidants, such as polyphenols, reduced MDA levels in broilers and piglets in muscle, liver and plasma (Goñi *et al*., 2007, Luehring *et al*., 2011, and Gerasopoulos *et al*., 2015, respectively). In the present study heat stress reduced serum MDA concentrations on d 2, but it was increased on d 28. These results suggest that the heat stress may not have been severe enough to promote peroxidation of cellular lipids when measured on d 2, but that prolonged heat stress caused oxidative stress. Thus the reduction of MDA concentrations during short-term heat stress (d 2) suggested that the enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic (vitamin A and vitamin E) antioxidant system reacted effectively against oxidation, but that this could not be maintained during prolonged heat stress (Pisoschi and Pop, 2015). Supplementation of vitamin E in feed or in water resulted in greater MDA concentrations in serum when compared to the other treatments, which is contrary to expectations. Other studies found that vitamin E and polyphenol-based antioxidants did not affect MDA concentrations in loin muscle in finishing pigs and diabetic or not diabetic rats (Hasty *et al*., 2002 and Belviranli *et al*., 2012).

In the present study, no effects on MDA concentrations in the ileum due to heat stress or supplementation were observed. Also, MDA was increased with dietary vitamin E and polyphenol supplementation under heat stress, but MDA was reduced with vitamin E supplementation in water. Lambert *et al*. (2002) reported no increase in lipid peroxidation products in the small

intestine of rats under high temperatures (42.5ºC). Contrarily, Maini *et al*. (2007) found that adding 200 IU dietary vitamin E to diets fed to broilers under heat stress reduced MDA concentrations due to amelioration of enzymatic and nonenzymatic antioxidant system by the vitamin E. On the other hand, Ebrahimzadeh *et al*. (2018) showed a greater reduction in MDA levels when using polyphenols (7.5 % of grape pomace) than vitamin E (200 mg of α-tocopherol acetate/ kg feed) in broilers.

Tight junctions (TJ) provide structural integrity and barrier function in the epithelium. When the TJ are dysregulated by heat stress, it causes alteration in the barrier function, producing pro-inflammatory and anti-inflammatory cytokines (Bhat *et al*., 2018). Thus, under heat stress, the pro-inflammatory cytokine TNF-α is produced (Bouchama *et al*., 1991). In the present study, TNF-α in the ileum and jejunum was reduced by the heat-stressed environment. We can speculate that the reduction of TNF-α in ileum and jejunum under heat stress can be due to the inhibition of NF-κB or the peak of TNF-α occurred before the d 28 collection day. It can be supported by the fact that TNF-α, when activated, is responsible for febrile responses (Cannon *et al*., 1990).

Bouchama *et al*. (1991) and Liu *et al*. (2016) did not find significant changes in TNF-α in jejunum and ileum of pigs housed under heat stress (35ºC) when using dietary vitamin E and selenium. In the present study, supplementation of vitamin E in feed and in water resulted in a reduction of TNF-α when compared to the rest of the dietary treatments for pigs housed under heat stress. This may suggest that dietary supplementation with vitamin E reduced some inflammation in tissue of pigs during heat stress.

Serum TNF-α, IL-1α, IL-1β, IL-2, IL-4, IL-6, and IL-10 were not affected by heat stress, supplementation or day of measurement. Perhaps, the heat-stressed environment was not enough to produce inflammation in the body. In contrast, for serum TNF-α, Gabler *et al*. (2018) reported

significantly lower serum TNF-α levels in pigs under heat stress on d 3. Likewise, Pearce *et al*. (2015) showed a reduction in TNF-α in serum of growing pigs under heat stress due to the inhibition of nuclear factor kappa-light-chain enhance of activated B cell (NF-κB) by heat shock proteins produced by heat stress. Heat exposure reduced TNF-α in the ileum suggesting that heat stress had effects at the local tissue level and probably could not be detected in serum. Thus, the expression of mucosal TNF-α can be different than circulating TNF-α (Cannon *et al*., 1990; Pearce *et al*., 2013). Similarly, TNF-α concentrations in serum were not affected by heat exposure for increasing duration (0, 2, 4 and 6 h) in finishing pigs (Pearce *et al*., 2014).

In the present study, serum IFN-γ concentration increased on d 28 but IL-12 and IL-18 were reduced on d 28 compared to d 2, showing low inflammatory responses, even though IL-12 and IL-18 act synergistically inducing IFN-γ (McInnes, 2017). Additionally, IL-8, a pro-inflammatory cytokine and activator of neutrophils in local inflammation (Baggiolini and Clark-Lewis, 1992) was reduced in serum by heat stress and increased on d 28 compared to d 2. In contrast, Liu *et al*. (2016) did not observe changes in IL-8 in the jejunum and ileum of 20-kg pigs when exposed to 20ºC or 35ºC using dietary vitamin E and selenium. IL-1ra is a natural anti-inflammatory cytokine protein which increases during inflammation (Arend *et al*., 1998) and has an antagonist effect on IL-1β and IL-1α (McInnes, 2017). In the present study, serum IL-1ra increased due to heat exposure, and IL-1ra was reduced on d 28 compared to d 2. Based on this result, heat exposure produced some inflammation, increasing IL-1ra in serum to counteract this inflammation in the pigs. Also, the reduction of IL-1ra on d 28 suggests the early potential presence of injurious components in the body (Artis, 2008) with the following resolution by d 28.

Red blood cell count, hemoglobin and hematocrit percentage rise or fall altogether, and increase due to deprivation of drinking water or decrease due to blood loss (Whalan, 2015). Red

blood cells have high polyunsaturated fatty acids in their membranes and can be affected by oxidative stress and serving their high concentrations of oxygen as ROS precursors (Cicha *et al*., 1999). In the present study, red blood cell count, hemoglobin and hematocrit percentage were reduced by 1.5, 3.0 and 3.7%, respectively by the heat stress environment on d 28. Likewise, Mendoza *et al*. (2017) observed a small reduction of 1% in red blood cells, hemoglobin and hematocrit due to heat stress in 39-kg BW pigs. Also, Adenkola *et al*., (2011) showed a reduction of 19% in red blood cells during thermally stressful environmental conditions in adult pigs by 3 months (harmattan season). Thus, in the present study, the reduction of red blood cell count, hemoglobin and hematocrit in the heat-stressed environment at d 28 could be associated with the oxidation of polyunsaturated fatty acids in the red blood cells by heat stress (Cicha *et al*. 1999) and impaired synthesis of hemoglobin (Habibu *et al*., 2018)., Even though the heat stressed pigs had reduced ingestion of water and possibly dehydration, this fact was not significant enough to elevate red blood cell count, hemoglobin and hematocrit. All CBC values were within normal ranges (Thorn, 2006).

White blood cells play a critical role in the immune system. Within the white blood cells, there are neutrophils, monocytes, lymphocytes, eosinophils, and basophils (Whalan, 2015). In the present study, white blood cells, neutrophils, and monocytes were not affected by heat stress, but they were decreased on d 28 by 29%, 75%, and 69%, respectively, compared to d 2. On the other hand, Mendoza *et al*. (2017) reported reductions in neutrophils (-10 %) due to heat stress. Adenkola *et al*. (2011) reported increased numbers of white blood cells, neutrophils, but no differences in monocytes, during the hot-dry season (temperatures between 30-34ºC) in adult pigs. In the present study, the reduction in white blood cells, neutrophils, and monocytes on d 28 can be

due to a resolution of a potential injury in the pigs, even though values were within normal ranges (Thorn, 2006).

Platelets are involved in aggregation and clot formation and immunity (Whalan, 2015 and Habibu *et al*., 2018). Habibu *et al*. (2018) reported a reduction in platelet count due to heat stress in cattle and ducks. In the present study, platelets were reduced by 40% on d 28. Probably this reduction was caused by resolution of a potential injury in the body, but the total values were 29% below the normal range (Thorn, 2006).

In the present study, the supplementation of vitamin E in feed and in water, and dietary polyphenol did not affect red blood cells, hemoglobin, hematocrit, white blood cells, neutrophils, monocytes and platelets. Attia *et al*. (2017) did not find significant differences in complete blood count when dietary vitamin E was supplemented in the feed of broilers under heat stress. Likewise, Stukelj *et al*. (2010) did not observe changes in hematological parameters of 7-weeks pigs when dietary polyphenols were supplemented in the diet.

In conclusion, heat stress reduced BW, ADFI, ADG and G:F, but the supplementation of vitamin E in water or dietary vitamin E or dietary polyphenol did not impact growth performance during heat stress. Heat stress increased rectal temperature and respiration rate, but this was not affected by antioxidant supplementation. Intestinal morphology was not affected by heat stress or by antioxidant supplementation, even though cellular proliferation increased with dietary vitamin E and vitamin E supplementation in water. Serum and liver concentrations of vitamin E increased with vitamin E supplementations, but not polyphenols. Serum MDA increased with antioxidant supplementations and dietary vitamin E and polyphenol increased MDA in ileum under heat stress. Cytokines in serum were no altered by heat stress or antioxidant supplementation, but TNF-α was decreased in jejunum and ileum by vitamin E supplementation in water. Red blood cells,

hemoglobin and hematocrit percentage were reduced by heat stress on d 28. The addition of the antioxidants in feed or in drinking water did not ameliorate the negative effects caused by heat stress on growing pigs.

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**References**

Adenkola, A. Y., Ayo, J. O., & Asala, O. O. (2011). Variations in haematological parameters and erythrocyte osmotic fragility of pigs during hot-dry and harmattan season in northern guinea savanna zone of nigeria. *Nigerian Journal of Physiological Sciences: Official Publication of the Physiological Society of Nigeria, 26*(2), 113-118.

Almeida, J. S., Iriabho, E. E., Gorrepati, V. L., Wilkinson, S. R., Grüneberg, A., Robbins, D. E., & Hackney, J. R. (2012). Personalized, participated, pervasive, and reproducible image bioinformatics in the web browser. *Journal of Pathology Informatics, 3*(1), 25. doi:10.4103/2153-3539.98813

AOAC (2006) Official Methods of Analysis. 18th Edition, Association of Official Analytical Chemists, Gaithersburgs, MD.

Arend, W. P., Malyak, M., Guthridge, C. J., & Gabay, C. (1998). Interleukin-1 receptor antagonist: Role in biology. *Annual Review of Immunology, 16*(1), 27-55. doi:10.1146/annurev.immunol.16.1.27

Artis, D. (2008). Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nature Reviews Immunology, 8*(6), 411-420. doi:10.1038/nri2316

Attia, Y. A., Al-Harthi, M. A., El-Shafey, A. S., Rehab, Y. A., & Kim, W. K. (2017). Enhancing tolerance of broiler chickens to heat stress by supplementation with vitamin E, vitamin C and/or probiotics. *Annals of Animal Science, 17*(4), 1155-1169. doi:10.1515/aoas-2017-0012

Augustin, K., Blank, R., Boesch-Saadatmandi, C., Frank, J., Wolffram, S., & Rimbach, G. (2008). Dietary green tea polyphenols do not affect vitamin E status, antioxidant capacity and meat

quality of growing pigs. *Journal of Animal Physiology and Animal Nutrition, 92*(6), 705-711. doi:10.1111/j.1439-0396.2007.00768.x

Baggiolini, M., & Clark-Lewis, I. (1992). *Interleukin-8, a chemotactic and inflammatory cytokine*. England: Elsevier B.V. doi:10.1016/0014-5793(92)80909-Z

Belviranli, M., Gökbel, H., Okudan, N., & Büyükba, S. \K. (2012). Oxidative stress and anti-oxidant status in diabetic rat liver: Effect of plant polyphenols. *Archives of Physiology and Biochemistry, 118*(5), 237-243. doi:10.3109/13813455.2012.702775

Bhat, A. A., Uppada, S., Achkar, I. W., Hashem, S., Yadav, S. K., Shanmugakonar, M., Al-Naemi, H. A., Mohammad H., & Uddin, S. (2018). Tight junction proteins and signaling pathways in cancer and inflammation: A functional crosstalk. *Frontiers in Physiology, 9,* 1942. doi:10.3389/fphys.2018.01942

Bollengier-Lee, S. (1999). Optimal dietary concentration of vitamin E for alleviating the effect of heat stress on egg production in laying hens. *British Poultry Science, 40*(1), 102-107. doi:10.1080/00071669987917

Bouchama, A., Parhar, R. S., el-Yazigi, A., Sheth, K. & al-Sedairy, S. (1991). Endotoxemia and release of tumor necrosis factor and interleukin 1 alpha in acute heatstroke. *Journal of Applied Physiology, 70*(6), 2640-2644. doi:10.1152/jappl.1991.70.6.2640

Cannon, J.G., Tompkins, R. G., Gelfand, J. A., Michie, H. R., Stanford, G. G., van der Meer, J. W. M., Endres, S., Lonnemann, G., Corsetti, J., Chernow, B., Wilmore, D. W., Wolff, S. M., Burke, J. F., & Dinarello, C. A. (1990). Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *The Journal of Infectious Diseases, 161*(1), 79-84. doi:10.1093/infdis/161.1.79

Cervantes, M., Antoine, D., Valle, J. A., Vásquez, N., Camacho, R. L., Bernal, H., & Morales, A. (2018). Effect of feed intake level on the body temperature of pigs exposed to heat stress conditions. *Journal of Thermal Biology, 76*, 1-7. doi:10.1016/j.jtherbio.2018.06.010

Chow, C. K. (1991). *Vitamin E and oxidative stress* doi://doi.org/10.1016/0891-5849(91)90174-2

Cicha, I., Suzuki, Y., Tateishi, N., & Maeda, N. (1999). Rheological changes in human red blood cells under oxidative stress. *Pathophysiology, 6*(2), 103-110. doi:10.1016/S0928-4680(99)00005-X

Collin, A., van Milgen, J., Dubois, S., & Noblet, J. (2001). Effect of high temperature and feeding level on energy utilization in piglets. *Journal of Animal Science, 79*(7), 1849-1857. doi:10.2527/2001.7971849x

Ebrahimzadeh, S. K., Navidshad, B., Farhoomand, P., & Mirzaei Aghjehgheshlagh, F. (2018). Effects of grape pomace and vitamin E on performance, antioxidant status, immune response, gut morphology and histopathological responses in broiler chickens. *South African Journal of Animal Science, 48*(2), 324-336. doi:10.4314/sajas.v48i2.13

Gabler, N. K., Koltes, D., Schaumberger, S., Murugesan, G. R., & Reisinger, N. (2018). Diurnal heat stress reduces pig intestinal integrity and increases endotoxin translocation. *Translational Animal Science, 2*(1), 1-10. doi:10.1093/tas/txx003

Gerasopoulos, K., Stagos, D., Petrotos, K., Kokkas, S., Kantas, D., Goulas, P., & Kouretas, D. (2015). *Feed supplemented with polyphenolic byproduct from olive mill wastewater processing improves the redox status in blood and tissues of piglets* doi://doi.org/10.1016/j.fct.2015.11.007

Gessner, D. K., Fiesel, A., Most, E., Dinges, J., Wen, G., Ringseis, R., & Eder, K. (2013). Supplementation of a grape seed and grape marc meal extract decreases activities of the oxidative stress-responsive transcription factors NF-κB and Nrf2 in the duodenal mucosa of pigs. *Acta Veterinaria Scandinavica, 55*(1), 18. doi:10.1186/1751-0147-55-18

Goñi, I., Brenes, A., Centeno, C., Viveros, A., Saura-Calixto, F., Rebolé, A., Arija, I., & Estevez, R. (2007). Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens. *Poultry Science, 86*(3), 508-516. doi:10.1093/ps/86.3.508

Grotto, D., Maria, L. S., Valentini, J., Paniz, C., Schmitt, G., Garcia, S. C., Pomblum, V. J., Rocha, J. B. T., & Farina, M. (2009). Importance of the lipid peroxidation biomarkers and methodological aspects FOR malondialdehyde quantification. *Química Nova, 32*(1), 169-174. doi:10.1590/S0100-40422009000100032

Habibu, B., Dzenda, T., Ayo, J. O., Yaqub, L. S., & Kawu, M. U. (2018). Haematological changes and plasma fluid dynamics in livestock during thermal stress, and response to mitigative measures. *Livestock Science, 214*, 189-201. doi:10.1016/j.livsci.2018.05.023

Hasty, J. L., van Heugten, E., See, M. T., & Larick, D. K. (2002). Effect of vitamin E on improving fresh pork quality in berkshire- and hampshire-sired pigs. *Journal of Animal Science, 80*(12), 3230-3237. doi:10.2527/2002.80123230x

Kerr, B. J., Yen, J. T., Nienaber, J. A., Easter, R. A. (2003). Influences of dietary protein level, amino acid supplementation and environmental temperature on performance, body composition, organ weights and total heat production of growing pigs, *Journal of Animal Science*, Volume 81, Issue 8, pp 1998–2007, doi.org/10.2527/2003.8181998x

Kim, B. G., and M. D. Lindemann. (2007). A spreadsheet method for experimental animal allotment. J. Anim. Sci.85 (Suppl. 2):112.

Kirunda, D. F., Scheideler, S. E., & McKee, S. R. (2001). The efficacy of vitamin E (DL - tocopheryl acetate) supplementation in hen diets to alleviate egg quality deterioration associated with high temperature exposure. *Poultry Science, 80*(9), 1378-1383. doi:10.1093/ps/80.9.1378

Kurz, A., M.D. (2008). Physiology of thermoregulation. *Best Practice & Research: Clinical Anaesthesiology, 22*(4), 627-644. doi:10.1016/j.bpa.2008.06.004

Lambert, G. P., Gisolfi, C. V., Berg, D. J., Moseley, P. L., Oberley, L. W., & Kregel, K. C. (2002). Molecular biology of thermoregulation: Selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. *Journal of Applied Physiology, 92*(4), 1750. doi:10.1152/japplphysiol.00787.2001

Liu, F., Cottrell, J. J., Furness, J. B., Rivera, L. R., Kelly, F. W., Wijesiriwardana, U., Pustovit., R. V., Fothergill, L. J., Bravo, D. M., Celi, P., Leury, B. J., Gabler, N. K., & Dunshea, F. R. (2016). Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. *Experimental Physiology, 101*(7), 801-810. doi:10.1113/EP085746

Luehring, M., Blank, R., & Wolffram, S. (2011). Vitamin E-sparing and vitamin E-independent antioxidative effects of the flavonol quercetin in growing pigs. *Animal Feed Science and Technology, 169*(3-4), 199-207. doi:10.1016/j.anifeedsci.2011.06.006

Maini, S., Rastogi, S. K., Korde, J. P., Madan, A. K., & Shukla, S. K. (2007). Evaluation of oxidative stress and its amelioration through certain antioxidants in broilers during summer. The Journal of Poultry Science44 (3), 339-347. doi.org/10.2141/jpsa.44.339

McInnes, I. B. (2017). In Firestein G. S., Budd R. C., Gabriel S. E., McInnes I. B. and O'Dell J. R. (Eds.), *Chapter 26 - cytokines* Elsevier. doi://doi.org/10.1016/B978-0-323-31696-5.00026-7

Mendoza, S.M., Boyd, R. D., Ferket, P. R., & van Heugten, E. (2017). Effects of dietary supplementation of the osmolyte betaine on growing pig performance and serological and hematological indices during thermoneutral and heat-stressed conditions 1. *Journal of Animal Science, 95*(11), 5040-5053. doi:10.2527/jas2017.1905

Montilla, S. I. R., Johnson, T. P., Pearce, S. C., Gardan-Salmon, D., Gabler, N. K., Ross, J. W., Ross, J. W., Rhoads, R. P., Baumgard, L. H., Lonergan, S. M. & Selsby, J. T. (2014). Heat stress causes oxidative stress but not inflammatory signaling in porcine skeletal muscle. *Temperature, 1*(1), 42-50. doi:10.4161/temp.28844

Niu, Z. Y., Liu, F. Z., Yan, Q. L., & Li, W. C. (2009). Effects of different levels of vitamin E on growth performance and immune responses of broilers under heat stress. *Poultry Science, 88*(10), 2101-2107. doi:10.3382/ps.2009-00220

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Oliver, S.R., Phillips, N. A., Novosad, V. L., Bakos, M. P., Talbert, E. E., & Clanton, T. L. (2012). Hyperthermia induces injury to the intestinal mucosa in the mouse: Evidence for an oxidative stress mechanism. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 302*(7), 845-853. doi:10.1152/ajpregu.00595.2011

Patience, J. F., Umboh, J. F., Chaplin, R. K., & Nyachoti, C. M. (2005). Nutritional and physiological responses of growing pigs exposed to a diurnal pattern of heat stress. *Livestock Production Science, 96*(2), 205-214. doi:10.1016/j.livprodsci.2005.01.012

Pearce, S. C., Mani, V., Boddicker, R. L., Johnson, J. S., Weber, T.E., Ross, J.W., Baumgard, L.H., Gabler, N. K. (2013). Heat stress reduces intestinal barrier integrity and favors intestinal glucose transport in growing pigs. *PLoS One, 8*(8), e70215. doi:10.1371/journal.pone.0070215

Pearce, S. C., Sanz-Fernandez, M. V., Hollis, J. H., Baumgard, L. H., & Gabler, N. K. (2014). Short term exposure to heat stress attenuates appetite and intestinal integrity in growing pigs. *Journal of Animal Science, 92*(12), 5444. doi:10.2527/jas.2014-8407

Pearce, S. C., Sanz Fernandez, M., Torrison, J., Wilson, M. E., Baumgard, L. H., & Gabler, N. K. (2015). Dietary organic zinc attenuates heat stress–induced changes in pig intestinal integrity and metabolism. *Journal of Animal Science, 93*(10), 4702-4713. doi:10.2527/jas.2015-9018

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry, 97*, 55-74. doi:10.1016/j.ejmech.2015.04.040

Quiniou, N., Dubois, S., & Noblet, J. (2000). Voluntary feed intake and feeding behaviour of group-housed growing pigs are affected by ambient temperature and body weight. *Livestock Production Science, 63*(3), 245-253. doi:10.1016/S0301-6226(99)00135-9

Quiniou, N., & Noblet, J. (1999). Influence of high ambient temperatures on performance of multiparous lactating sows. *Journal of Animal Science, 77*(8), 2124. doi:10.2527/1999.7782124x

Renaudeau, D., Anais, C., Tel, L., & Gourdine, J. L. (2010). Effect of temperature on thermal acclimation in growing pigs estimated using a nonlinear function1. *Journal of Animal Science, 88*(11), 3715-3724. doi:10.2527/jas.2009-2169

Renaudeau, D., Gourdine, J. L., & St-Pierre, N. R. (2011). A meta-analysis of the effects of high ambient temperature on growth performance of growing-finishing pigs. *Journal of Animal Science, 89*(7), 2220-2230. doi:10.2527/jas.2010-3329

Reuter, S., Gupta, S. C., Chaturvedi, M. M., & Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer: How are they linked? *Free Radical Biology and Medicine, 49*(11), 1603-1616. doi:10.1016/j.freeradbiomed.2010.09.006

Sakaguchi, Y., Stephens, L. C., Makino, M., Kaneko, T., Strebel, F. R., Danhauser, L. L., Jenkins, G. N., & Bull, J. M. C. (1995). Apoptosis in tumors and normal tissues induced by whole body hyperthermia in rats. *Cancer Research, 55*(22), 5459-5464.

Sanz Fernandez, M. V., Johnson, J. S., Abuajamieh, M., Stoakes, S. K., Seibert, J. T., Cox, L., Kahl, S., Elsasser, T. H., Ross, J. W., Isom, S. C., Rhoads, R. P., Baumgard, L. H. 2015. Effects of heat stress on carbohydrate and lipid metabolism in growing pigs, *Physiol Rep*, 3 ( 2), 2015, e12315, doi: 10.14814/phy2.12315

Sies, H., Stahl, W., & Sundquist, A. R. (1992). Antioxidant functions of vitamins. *Annals of the New York Academy of Sciences, 669*(1), 7-20. doi:10.1111/j.1749-6632.1992.tb17085.x

Smith, M. J., Inserra, P. F., Watson, R. R., Wise, J. A., & O'Neill, K. L. (1999). Supplementation with fruit and vegetable extracts may decrease DNA damage in the peripheral lymphocytes of an elderly population. *Nutrition Research, 19*(10), 1507-1518. doi:10.1016/S0271-5317(99)00107-4

St-Pierre, N. R., Cobanov, B., & Schnitkey, G. (2003). Economic losses from heat stress by US livestock industries. *Journal of Dairy Science, 86*(13 Electronic Supplement 1), E77. doi:10.3168/jds.S0022-0302(03)74040-5

Stukelj, M., Valencak, Z., Krsnik, M., & Svete, A. N. (2010). The effect of the combination of acids and tannin in diet on the performance and selected biochemical, haematological and antioxidant enzyme parameters in grower pigs. *Acta Veterinaria Scandinavica, 52*(1), 19. doi:10.1186/1751-0147-52-19

Surai, P. F. (2014). Polyphenol compounds in the chicken/animal diet: From the past to the future. *Journal of Animal Physiology and Animal Nutrition, 98*(1), 19-31. doi:10.1111/jpn.12070

Thorn C. E. In: Chalm's Veterinary Hematology. Feldman BF, Zinkl GJ, Jain NC, editor. Denmark: Blackwell publishing, Narayana Press; 2006. Normal hematology of the pig; pp. 1089–1095.

Touchette, K. J., Carroll, J. A., Allee, G. L., Matteri, R. L., Dyer, C. J., Beausang, L. A., & Zannelli, M. E. (2002). Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. effects on the immune axis of weaned pigs1. *Journal of Animal Science, 80*(2), 494-501. doi:10.2527/2002.802494x

Vajrabukka, C., Thwaites, C. J., & Farrell, D. J. 1981. Overcoming the effects of high temperature on pig growth**.** Recent Advances in Animal Nutrition in Australia (1981), pp. 99-114

van Heugten, E., Sweet, L.A., Stumpf, T.T., Risley, C.R. and Schell, T.C. 1997. Effects of water supplementation with selenium and vitamin E on growth performance and blood selenium and serum vitamin E concentrations in weanling pigs. J. Am. Vet. Med. Assoc. 211:1039-1042.

Vergauwen, H., Tambuyzer, B., Jennes, K., Degroote, J., Wang, W., De Smet, S., Michiels, J. & Van Ginneken, C. (2015). Trolox and ascorbic acid reduce direct and indirect oxidative stress in the IPEC-J2 cells, an in vitro model for the porcine gastrointestinal tract. *PLoS One, 10*(3), e0120485. doi:10.1371/journal.pone.0120485

Verhelst, R., Schroyen, M., Buys, N., & Niewold, T. (2014). Dietary polyphenols reduce diarrhea in enterotoxigenic escherichia coli (ETEC) infected post-weaning piglets. *Livestock Science, 160,* 138-140. doi:10.1016/j.livsci.2013.11.026

Volodina, O., Ganesan, S., Pearce, S. C., Gabler, N. K., Baumgard, L. H., Rhoads, R. P., & Selsby, J. T. (2017). Short-term heat stress alters redox balance in porcine skeletal muscle. *Physiological Reports, 5*(8), n/a. doi:10.14814/phy2.13267

Whalan J.E. (2015) Hematology Highlights. In: A Toxicologist's Guide to Clinical Pathology in Animals. Springer, Cham

White, H. M., Richert, B. T., Schinckel, A. P., Burgess, J. R., Donkin, S. S., & Latour, M. A. (2008). Effects of temperature stress on growth performance and bacon quality in grow-finish pigs housed at two densities. *Journal of Animal Science, 86*(8), 1789-1798. doi:10.2527/jas.2007-0801

Wilburn, E. E., Mahan, D. C., Hill, D. A., Shipp, T. E., & Yang, H. (2008). An evaluation of natural (RRR-α-tocopheryl acetate) and synthetic (all-rac-α-tocopheryl acetate) vitamin E fortification in the diet or drinking water of weanling pigs. *Journal of Animal Science, 86*(3), 584-591. doi:10.2527/jas.2007-0377

Yu, J., Yin, P., Liu, F., Cheng, G., Guo, K., Lu, A., Xiaoyu, Z., Luan, W & Xu, J. (2010). Effect of heat stress on the porcine small intestine: A morphological and gene expression

study. *Comparative Biochemistry and Physiology, Part A, 156*(1), 119-128. doi:10.1016/j.cbpa.2010.01.008

Zhang, H. J., Jiang, X. R., Mantovani, G., Lumbreras, A. E. V., Comi, M., Alborali, G., Savoini, G., Dell'Orto, V. & Bontempo, V. (2014). Modulation of plasma antioxidant activity in weaned piglets by plant polyphenols. *Italian Journal of Animal Science, 13*(2), ijas.2014.3242. doi:10.4081/ijas.2014.3242

Zhang, H., & Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science, 8,* 33-42. doi:10.1016/j.cofs.2016.02.002

**Table 1.** Composition of experimental diets. As-fed basis [1]

| | Treatments | | | |
|---|---|---|---|---|
| Item | **CON** | **VEW[2]** | **VEF** | **POL** |
| **Ingredient, %** | | | | |
| Corn, yellow dent | 75.63 | 75.63 | 75.63 | 75.63 |
| Soybean meal, 47.5% CP | 19.46 | 19.46 | 19.46 | 19.46 |
| Poultry fat | 1.74 | 1.74 | 1.74 | 1.74 |
| L-lysine HCL | 0.34 | 0.34 | 0.34 | 0.34 |
| DL-methionine | 0.07 | 0.07 | 0.07 | 0.07 |
| L-threonine | 0.11 | 0.11 | 0.11 | 0.11 |
| Monocalcium phosphate 21% | 1.01 | 1.01 | 1.01 | 1.01 |
| Limestone | 1.05 | 1.05 | 1.05 | 1.05 |
| Salt | 0.4 | 0.4 | 0.4 | 0.4 |
| Market swine vitamin[3] | 0.04 | 0.04 | 0.04 | 0.04 |
| Market swine minerals[4] | 0.15 | 0.15 | 0.15 | 0.15 |
| Vitamin E, IU/kg[5] | - | - | 200 | - |
| Polyphenols, mg/kg | - | - | - | 400 |
| Calculated ME Mcal/kg | 3300 | 3300 | 3300 | 3300 |
| SID Lys g: ME Mcal | 2.78 | 2.78 | 2.78 | 2.78 |
| **Analyzed composition[6]** | | | | |
| Crude protein, % | 15.39 | 15.72 | 15.60 | 16.20 |
| Moisture, % | 12.15 | 11.95 | 11.98 | 12.07 |
| Crude fat, % | 4.25 | 4.21 | 4.35 | 4.37 |
| Crude fiber, % | 2.17 | 2.12 | 2.34 | 2.39 |
| Ash, % | 3.96 | 4.24 | 4.42 | 4.12 |
| NDF, % | 8.74 | 8.05 | 8.44 | 8.99 |
| ADF, % | 3.39 | 3.22 | 3.63 | 3.42 |
| Ca, % | 0.63 | 0.68 | 0.75 | 0.69 |
| P, % | 0.55 | 0.62 | 0.60 | 0.61 |

[1]Diets were formulated to meet or exceed NRC (2012) recommendation for 41 to 75 kg pigs. Dietary treatments consisted of control diets (CON), vitamin E supplementation in water (VEW), vitamin E supplementation in feed (VEF), and polyphenol supplementation in feed (POL)

[2]Vitamin E in the drinking water was supplied as d-α-tocopherol (Emcelle® tocopherol, Stuart products, Bedford, TX).

[3]Supplied per kg of complete diet: 6,614 IU of vitamin A, 1,323 IU of vitamin $D_3$, 27 IU of vitamin E and 2.7 mg of vitamin K, 35 µg vitamin $B_{12}$, 6.2 mg riboflavin, 20 mg pantothenic acid, 35 mg niacin, and 0.09 mg biotin/

[3]Supplied per kg of complete diet: 33 ppm of manganese, 110 ppm of zinc, 110 ppm of iron, 17 ppm of copper, 0.30 ppm of iodine, and 0.30 ppm of selenium.

[5]Supplied as dl-α-tocopheryl acetate (Rovimix®, DSM, Heerlen, The Netherlands)

[6]Analyzed by Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO

**Table 2.** Growth performance of pigs exposed to thermo-neutral and heat-stressed environments and provided antioxidants in feed or water[1]

| | Environment[2] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Thermo-neutral | | | | Heat-stressed | | | | | | | |
| | Supplementation | | | | | | | | SEM | *P*-value[3] | | |
| Item | CON | VEW | VEF | POL | CON | VEW | VEF | POL | | E | S | E x S |
| Body weight, kg | | | | | | | | | | | | |
| d-7 | 46.9 | 47.3 | 47.4 | 47.1 | 47.4 | 47.6 | 47.4 | 47.2 | 0.972 | 0.889 | 0.677 | 0.845 |
| d0 | 52.8 | 54.0 | 52.5 | 52.9 | 53.8 | 54.2 | 53.1 | 53.3 | 1.099 | 0.711 | 0.096 | 0.885 |
| d7 | 60.8 | 61.4 | 59.9 | 60.6 | 58.2 | 59.4 | 58.8 | 58.7 | 1.243 | 0.217 | 0.485 | 0.766 |
| d14 | 68.2 | 67.7 | 67.9 | 68.5 | 64.1 | 65.9 | 64.3 | 64.5 | 1.439 | 0.060 | 0.868 | 0.540 |
| d21 | 75.8 | 76.5 | 75.5 | 76.7 | 69.5 | 71.2 | 70.5 | 70.2 | 1.574 | 0.004 | 0.653 | 0.853 |
| d28 | 82.4 | 83.2 | 81.6 | 83.6 | 74.3 | 76.4 | 75.3 | 75.1 | 1.733 | 0.001 | 0.465 | 0.715 |
| ADG, g/d | | | | | | | | | | | | |
| d-7 to d0 | 843 | 940 | 735 | 800 | 910 | 931 | 816 | 879 | 60.13 | 0.176 | 0.066 | 0.855 |
| d0 to d7 | 1143 | 1061 | 1049 | 1111 | 631 | 754 | 820 | 770 | 54.57 | <0.001 | 0.711 | 0.051 |
| d7 to d14 | 1049 | 897 | 1136 | 1109 | 838 | 921 | 783 | 832 | 91.14 | 0.035 | 0.863 | 0.085 |
| d14 to d21 | 1095 | 1264 | 1086 | 1171 | 777 | 761 | 873 | 813 | 70.00 | <0.001 | 0.708 | 0.213 |
| d21 to d28 | 935 | 954 | 875 | 972 | 672 | 745 | 690 | 699 | 57.79 | <0.001 | 0.582 | 0.817 |
| d0 to d28 | 1056 | 1043 | 1036 | 1092 | 729 | 795 | 795 | 779 | 36.62 | <0.001 | 0.554 | 0.370 |
| ADFI, kg/d (as-fed basis) | | | | | | | | | | | | |
| d-7 to d0[4] | 2.10 | 2.10 | 1.83 | 1.94 | 2.03 | 2.14 | 2.04 | 2.09 | 0.076 | 0.181 | 0.077 | 0.207 |
| d0 to d7 | 2.57 | 2.68 | 2.46 | 2.59 | 1.79 | 1.92 | 2.04 | 1.82 | 0.103 | <0.001 | 0.663 | 0.206 |
| d7 to d14 | 2.71 | 2.61 | 2.71 | 2.85 | 1.94 | 2.08 | 2.01 | 2.02 | 0.129 | <0.001 | 0.759 | 0.577 |
| d14 to d21 | 2.78 | 2.96 | 2.77 | 2.85 | 1.99 | 2.09 | 2.27 | 2.19 | 0.118 | <0.001 | 0.440 | 0.309 |
| d21 to d28 | 2.39 | 2.57 | 2.33 | 2.57 | 1.84 | 1.90 | 1.90 | 1.81 | 0.116 | <0.001 | 0.596 | 0.469 |
| d0 to d28 | 2.75 | 2.89 | 2.80 | 2.77 | 2.15 | 2.19 | 2.24 | 2.17 | 0.085 | <0.001 | 0.605 | 0.408 |
| G: F | | | | | | | | | | | | |
| d-7 to d0 | 0.393 | 0.450 | 0.399 | 0.404 | 0.448 | 0.438 | 0.397 | 0.416 | 0.024 | 0.388 | 0.302 | 0.533 |
| d0 to d7 | 0.441 | 0.396 | 0.431 | 0.431 | 0.331 | 0.385 | 0.403 | 0.418 | 0.023 | 0.030 | 0.219 | 0.076 |
| d7 to d14 | 0.37 | 0.27 | 0.42 | 0.37 | 0.44 | 0.45 | 0.37 | 0.42 | 0.048 | 0.189 | 0.707 | 0.048 |
| d14 to d21 | 0.396 | 0.439 | 0.394 | 0.417 | 0.396 | 0.358 | 0.407 | 0.384 | 0.028 | 0.326 | 0.998 | 0.256 |

**Table 2.** Continued

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| d21 to d28 | 0.398 | 0.344 | 0.378 | 0.379 | 0.363 | 0.395 | 0.365 | 0.392 | 0.025 | 0.793 | 0.927 | 0.396 |
| d0 to d28 | 0.383 | 0.364 | 0.372 | 0.393 | 0.339 | 0.362 | 0.353 | 0.362 | 0.011 | 0.002 | 0.386 | 0.505 |

[1] Values least square means of 16 pigs. Dietary treatments consisted of control diets (CON), vitamin E supplementation in water (VEW), vitamin E supplementation in feed (VEF), and polyphenol supplementation in feed (POL)
[2] Temperatures were set at the following time points: 0000, 0200, 0400, 0400, 0600, 0800, 1000, 1200, 1400, 1600, 1800, 2000, and 2200h. Temperatures for the thermo-neutral room were 18.9, 18.9, 20, 20, 21.1, 21.1, 22.2, 21.1, 21.1, 20, and 20°C, and for heat-stressed room they were 28.3, 29.4, 29.4, 31.1, 32.8, 33.3, 34.4, 35.6, 34.4, 31.7, 29.4 and 29.4°C.
[3] Effects abbreviations: E = environment, S = supplementation, E x S = environment x supplementation
[4]Dietary treatments were provided on d -7 and on environmental treatment started on d0.

**Table 3.** Intestinal histology and immunohistochemistry in pigs exposed to thermo-neutral and heat-stressed environments and provided antioxidants in feed or water[1]

| | Environment | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Thermo-neutral | | | | Heat-stressed | | | | | | | |
| | Supplementation | | | | | | | | | *P*-value[2] | | |
| Item | CON | VEW | VEF | POL | CON | VEW | VEF | POL | SEM | E | S | E x S |
| **Villus height, µm** | | | | | | | | | | | | |
| Jejunum | 425 | 381 | 464 | 402 | 368 | 359 | 378 | 394 | 28.898 | 0.119 | 0.368 | 0.514 |
| Ileum | 337 | 333 | 326 | 313 | 310 | 295 | 332 | 318 | 32.855 | 0.638 | 0.968 | 0.870 |
| **Villus width, µm** | | | | | | | | | | | | |
| Jejunum | 167 | 155 | 174 | 168 | 166 | 153 | 163 | 156 | 8.278 | 0.391 | 0.314 | 0.882 |
| Ileum | 156 | 175 | 160 | 163 | 152 | 162 | 176 | 182 | 10.955 | 0.686 | 0.253 | 0.286 |
| **Crypt depth, µm** | | | | | | | | | | | | |
| Jejunum | 148 | 134 | 138 | 150 | 130 | 134 | 106 | 131 | 10.989 | 0.133 | 0.300 | 0.516 |
| Ileum | 158 | 126 | 137 | 158 | 134 | 128 | 114 | 161 | 17.650 | 0.493 | 0.204 | 0.788 |
| **Villus/crypt ratio** | | | | | | | | | | | | |
| Jejunum | 2.92 | 3.08 | 3.39 | 2.77 | 2.90 | 2.81 | 3.66 | 3.08 | 0.233 | 0.652 | 0.046 | 0.645 |
| Ileum | 2.53 | 3.03 | 2.34 | 2.13 | 2.49 | 2.48 | 3.06 | 2.35 | 0.337 | 0.889 | 0.590 | 0.455 |
| **Enterocyte proliferation, Ki67[3]** | | | | | | | | | | | | |
| Jejunum, % | 43.7 | 38.4 | 41.9 | 41.2 | 48.1 | 47.7 | 48.1 | 45.6 | 2.305 | 0.037 | 0.534 | 0.660 |
| Ileum, % | 47.5 | 44.5 | 43.5 | 47.5 | 47.9 | 49.8 | 52.2 | 44.7 | 2.137 | 0.150 | 0.831 | 0.040 |

[1] Values least square means of 8 pigs. Dietary treatments consisted of control diets (CON), vitamin E supplementation in water (VEW), vitamin E supplementation in feed (VEF), and polyphenol supplementation in feed (POL)

[2] Effects abbreviations: E = environment, S = supplementation, E x S = environment x supplementation

[3] Proliferation was evaluated by staining crypt cells with ki67 antibody. Ki67 is a protein in the nucleus of proliferating cells

**Table 4.** Concentration of vitamin E in serum and liver of pigs exposed to thermo-neutral and heat-stressed environment and provided antioxidants in feed or water[1]

| | Environment | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Thermo-neutral | | | | Heat-stressed | | | | | | | | | | | |
| | Supplementation | | | | | | | | | *P*-value[2] | | | | | | |
| Item | CON | VEW | VEF | POL | CON | VEW | VEF | POL | SEM | E | S | D | E x D | E x S | S x D | E x S x D |
| **Vitamin E** Serum ppm[2], | | | | | | | | | | | | | | | | |
| Day 2 | 1.73 | 3.29 | 2.79 | 1.79 | 1.72 | 3.20 | 3.45 | 1.63 | 0.151 | 0.259 | <0.001 | 0.067 | 0.016 | 0.584 | 0.001 | 0.038 |
| Day 28 | 1.64 | 4.01 | 3.63 | 1.77 | 1.49 | 3.86 | 3.11 | 1.48 | | | | | | | | |
| **Vitamin E** Liver ppm[3], | 3.9 | 30.8 | 18.2 | 4.7 | 4.0 | 32.8 | 17.7 | 5.0 | 2.59 | 0.768 | <0.001 | - | - | 0.968 | - | - |

[1]Dietary treatments consisted of control diets (CON), vitamin E supplementation in water (VEW), vitamin E supplementation in feed (VEF), and polyphenol supplementation in feed (POL)
[2]Effects abbreviations: E = environment, S = supplementation, D = day, E x D = environment x day, Ex S = environment x supplementation, S x D = supplementation x day, E x S x D = environment x supplementation x day
[3]Values are least square means of 16 pigs
[4]Values are least square means of 8 pigs

**Table 5.** Oxidative stress status in serum and ileum mucosa of pigs exposed to thermo-neutral or heat-stressed environments and provided antioxidants in feed or water[1]

| | Environment | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Thermo-neutral | | | | Heat-stressed | | | | | | | | | |
| | Supplementation | | | | | | | | | *P*-value[2] | | | | |
| Item | CON | VEW | VEF | POL | CON | VEW | VEF | POL | SEM | E | S | D | E x D | E x S |
| MDA[2,3] serum µM, | | | | | | | | | | | | | | |
| Day 2 | 4.3 | 6.3 | 6.3 | 5.5 | 2.9 | 3.3 | 3.2 | 2.5 | 0.803 | 0.463 | 0.017 | <0.001 | <0.001 | 0.283 |
| Day 28 | 4.2 | 5.4 | 4.8 | 5.3 | 5.8 | 6.3 | 6.0 | 6.6 | | | | | | |
| MDA in mucosa, µmol/g protein[4] | | | | | | | | | | | | | | |
| Ileum | 0.362 | 0.208 | 0.185 | 0.159 | 0.180 | 0.183 | 0.225 | 0.229 | 0.040 | 0.538 | 0.151 | - | - | 0.005 |

[1]Dietary treatments consisted of control diets (CON), vitamin E supplementation in water (VEW), vitamin E supplementation in feed (VEF), and polyphenol supplementation in feed (POL).
[2]Effects abbreviations: E = environment, S = supplementation, D = day, E x D = environment x day, E x S = environment x supplementation, S x D= supplementation x day ($P$ = 0.418 for MDA serum), E x S x D= environment x supplementation x day ($P$ = 0.829 for MDA serum)
[3]MDA: malondialdehyde
[4]Values are least square means of 16 pigs
[5]Values are least square means of 8 pigs

**Table 6.** Immune markers in serum and intestinal mucosa of pigs exposed to thermo-neutral or heat-stressed environments and provided antioxidants in feed or water[1]

| | | Environment | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Thermo-neutral | | | | Heat-stressed | | | | | | | | | |
| | | Supplements | | | | | | | | | *P*–value[2] | | | | |
| Item | Day | CON | VEW | VEF | POL | CON | VEW | VEF | POL | SEM | E | S | D | E x S | E x S x D |
| Serum pg/mL[3], | | | | | | | | | | | | | | | |
| IFN-γ[4] | 2 | 3219 | 2608 | 2707 | 1332 | 2692 | 1531 | 3381 | 3622 | 1003.02 | 0.375 | 0.587 | 0.078 | 0.306 | 0.383 |
| | 28 | 2823 | 3686 | 3490 | 2888 | 5543 | 2496 | 3586 | 3759 | | | | | | |
| IL-1α[5] | 2 | 22 | 27 | 42 | 22 | 35 | 40 | 24 | 42 | 10.87 | 0.780 | 0.728 | 0.284 | 0.364 | 0.463 |
| | 28 | 15 | 25 | 36 | 34 | 25 | 29 | 28 | 21 | | | | | | |
| IL-1β[6] | 2 | 208 | 267 | 392 | 200 | 254 | 252 | 223 | 456 | 79.044 | 0.827 | 0.105 | 0.133 | 0.392 | 0.113 |
| | 28 | 136 | 210 | 210 | 346 | 200 | 128 | 260 | 301 | | | | | | |
| IL-1ra[7] | 2 | 738 | 672 | 690 | 456 | 953 | 814 | 716 | 846 | 104.75 | 0.056 | 0.871 | <0.001 | 0.274 | 0.615 |
| | 28 | 345 | 409 | 571 | 467 | 495 | 614 | 419 | 533 | | | | | | |
| IL-2[8] | 2 | 94 | 153 | 228 | 92 | 151 | 120 | 109 | 260 | 59.65 | 0.891 | 0.629 | 0.266 | 0.507 | 0.058 |
| | 28 | 54 | 145 | 102 | 189 | 131 | 72 | 159 | 103 | | | | | | |
| IL-4[9] | 2 | 525 | 730 | 1520 | 432 | 628 | 732 | 916 | 1592 | 438.15 | 0.801 | 0.375 | 0.217 | 0.906 | 0.059 |
| | 28 | 237 | 702 | 461 | 1127 | 562 | 325 | 1152 | 471 | | | | | | |
| IL-6[10] | 2 | 13 | 21 | 36 | 14 | 21 | 16 | 21 | 34 | 11.35 | 0.924 | 0.449 | 0.281 | 0.813 | 0.388 |
| | 28 | 5 | 14 | 28 | 20 | 11 | 21 | 24 | 9 | | | | | | |
| IL-8[11] | 2 | 428 | 450 | 377 | 410 | 318 | 344 | 292 | 343 | 74.767 | 0.007 | 0.359 | <0.001 | 0.282 | 0.508 |
| | 28 | 688 | 574 | 679 | 652 | 394 | 415 | 534 | 738 | | | | | | |
| IL-10[12] | 2 | 248 | 328 | 565 | 222 | 376 | 260 | 264 | 613 | 130.95 | 0.802 | 0.561 | 0.164 | 0.610 | 0.033 |
| | 28 | 177 | 270 | 223 | 408 | 300 | 169 | 402 | 240 | | | | | | |

**Table 6.** Continued

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IL-12[13] | 2 | 1224 | 1499 | 1526 | 1139 | 1324 | 1392 | 1346 | 1401 | 116.61 | 0.807 | 0.221 | 0.001 | 0.669 | 0.257 |
| | 28 | 1135 | 1153 | 1285 | 1183 | 1063 | 1274 | 1161 | 1057 | | | | | | |
| IL-18[14] | 2 | 803 | 924 | 1727 | 808 | 783 | 815 | 611 | 1141 | 231.83 | 0.324 | 0.482 | 0.002 | 0.250 | 0.023 |
| | 28 | 294 | 741 | 638 | 927 | 673 | 456 | 639 | 510 | | | | | | |
| TNF-α[15] | 2 | 78 | 57 | 69 | 78 | 83 | 46 | 43 | 59 | 31.969 | 0.920 | 0.983 | 0.576 | 0.968 | 0.802 |
| | 28 | 37 | 60 | 49 | 55 | 26 | 64 | 99 | 48 | | | | | | |
| TNF-α Jejunum, pg/g protein[16] | | 847 | 1094 | 786 | 517 | 412 | 1112 | 191 | 723 | 196.84 | 0.022 | 0.064 | - | 0.535 | - |
| Ileum, pg/mg protein[16] | | 5.6 | 6.4 | 2.3 | 2.4 | 1.5 | 2.1 | 2.0 | 2.8 | 0.452 | 0.009 | <0.001 | - | <0.001 | - |

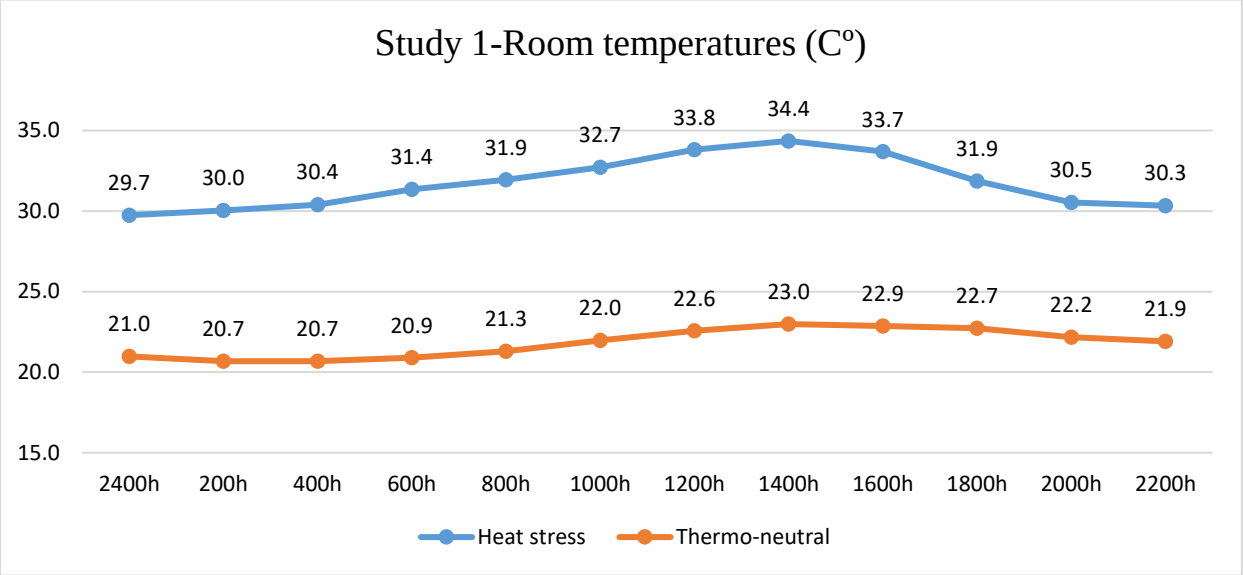
[1]Dietary treatments consisted of control diets (CON), vitamin E supplementation in water (VEW), vitamin E supplementation in feed (VEF), and polyphenol supplementation in feed (POL).
[2]Effects abbreviations: E = environment, S = supplementation, D = day, E x D = environment x day, E x S = environment x supplementation, S x D = supplementation x day, E x S x D = environment x supplementation x day. No interactions were reported on E x D = environment x day, and S x D = supplementation x day
[3]Values are least square means of 16 pigs
[4]IFN-γ: interferon-gamma
[5]IL-α: interleukin-alpha
[6]IL-β: interleukin-beta
[7]IL-1ra: interleukin-1receptor antagonist
[8]IL-2: interleukin-2
[9]IL-4: interleukin-4
[10]IL-6: interleukin-6
[11]IL-8: interleukin-8
[12]IL-10: interleukin-10
[13]IL-12: interleukin-12
[14]IL-18: interleukin-18
[15]TNF-α: tumor necrosis factor- alpha
[16]Values least square means of 8 pigs

**Table 7.** Complete blood count measured on d 2 and d 28 in pigs exposed to thermo-neutral or heat-stressed environments and provided antioxidants in feed or water[1]

| | | Environment | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Thermo-neutral | | | | Heat-stressed | | | | | | | | |
| | | Supplements | | | | | | | | | *P*-value[2] | | | |
| Item | Day | CON | VEW | VEF | POL | CON | VEW | VEF | POL | SEM | E | S | D | E x D |
| White blood cells, $10^3$/µL | 2 | 17.2 | 18.8 | 17.9 | 17.5 | 17.9 | 17.7 | 17.5 | 16.0 | 1.129 | 0.581 | 0.255 | <0.001 | 0.84 |
| | 28 | 14.8 | 13.3 | 14.1 | 12.8 | 13.8 | 13.8 | 13.4 | 12.5 | | | | | |
| Red blood cells, $10^6$/µL | 2 | 6.65 | 6.83 | 6.71 | 6.51 | 6.84 | 6.76 | 6.73 | 6.58 | 0.223 | 0.287 | 0.750 | 0.430 | 0.020 |
| | 28 | 6.94 | 6.94 | 6.80 | 7.33 | 6.57 | 6.70 | 6.42 | 6.56 | | | | | |
| Hemoglobin, g/dL | 2 | 12.14 | 12.45 | 12.37 | 11.97 | 12.21 | 12.34 | 12.25 | 12.21 | 0.366 | 0.107 | 0.563 | 0.188 | 0.003 |
| | 28 | 12.73 | 12.98 | 12.79 | 13.49 | 11.72 | 12.09 | 11.98 | 11.99 | | | | | |
| Hematocrit, % | 2 | 40.6 | 41.6 | 41.2 | 39.3 | 40.5 | 40.9 | 41.2 | 40.4 | 1.367 | 0.152 | 0.767 | 0.670 | 0.013 |
| | 28 | 42.0 | 42.1 | 41.8 | 44.8 | 39.0 | 40.2 | 38.5 | 39.5 | | | | | |
| Platelet Count, $10^3$/µL | 2 | 252 | 260 | 242 | 247 | 312 | 270 | 305 | 282 | 31.33 | 0.268 | 0.542 | <0.001 | 0.382 |
| | 28 | 219 | 169 | 180 | 170 | 205 | 217 | 219 | 175 | | | | | |
| Neutrophils, /µL | 2 | 7223 | 6559 | 6353 | 6401 | 6471 | 6430 | 5969 | 5221 | 572.3 | 0.247 | 0.215 | <0.001 | 0.658 |
| | 28 | 3908 | 3622 | 4426 | 3231 | 3336 | 3795 | 3525 | 3017 | | | | | |
| Lymphocytes, /µL | 2 | 8272 | 9503 | 9667 | 9269 | 9782 | 9528 | 9609 | 8972 | 731.4 | 0.795 | 0.758 | 0.123 | 0.652 |
| | 28 | 9786 | 8540 | 8462 | 8396 | 9164 | 8850 | 8676 | 8415 | | | | | |
| Monocytes, /µL | 2 | 1219 | 1256 | 1142 | 1226 | 1002 | 1121 | 1339 | 1289 | 117.99 | 0.647 | 0.889 | <0.001 | 0.768 |
| | 28 | 736 | 720 | 754 | 733 | 771 | 651 | 705 | 590 | | | | | |
| Eosinophils, /µL | 2 | 400 | 435 | 653 | 519 | 582 | 531 | 499 | 468 | 98.99 | 0.500 | 0.702 | 0.304 | 0.336 |
| | 28 | 348 | 412 | 458 | 422 | 534 | 535 | 502 | 497 | | | | | |

[1]Values are least square means of 16 pigs. Dietary treatments consisted of control diets (CON), vitamin E supplementation in water (VEW), vitamin E supplementation in feed (VEF), and polyphenol supplementation in feed (POL).
[2]Effects abbreviations: E = environment, S = supplementation, D = day, E x D = environment x day. Interactions on E x S = environment x supplementation, S x D = supplementation x day, and E x S x D = environment x supplementation x day were not reported on the count blood cells ($P \geq 0.05$)

**A**




**B**

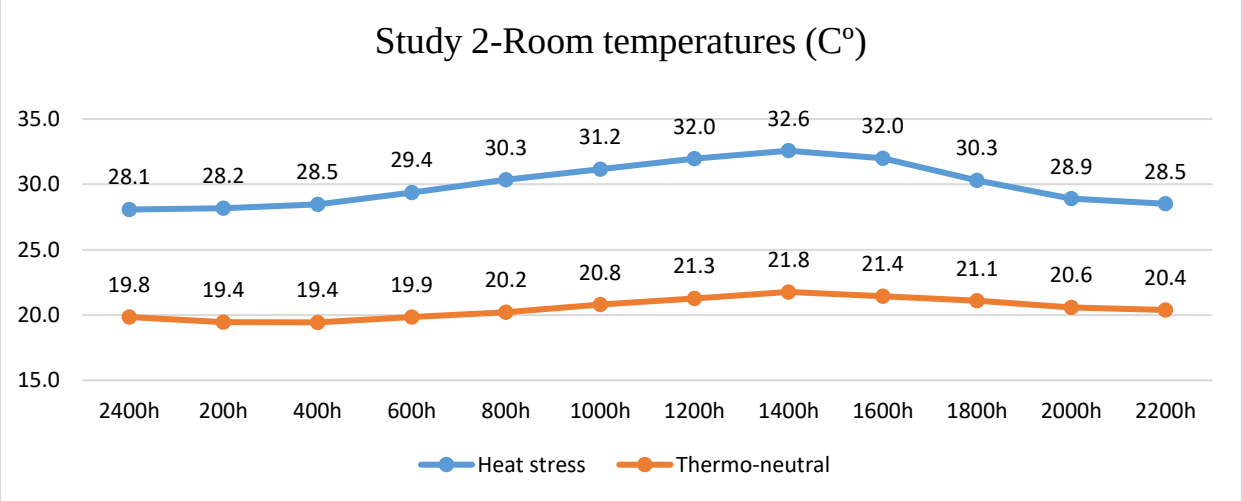
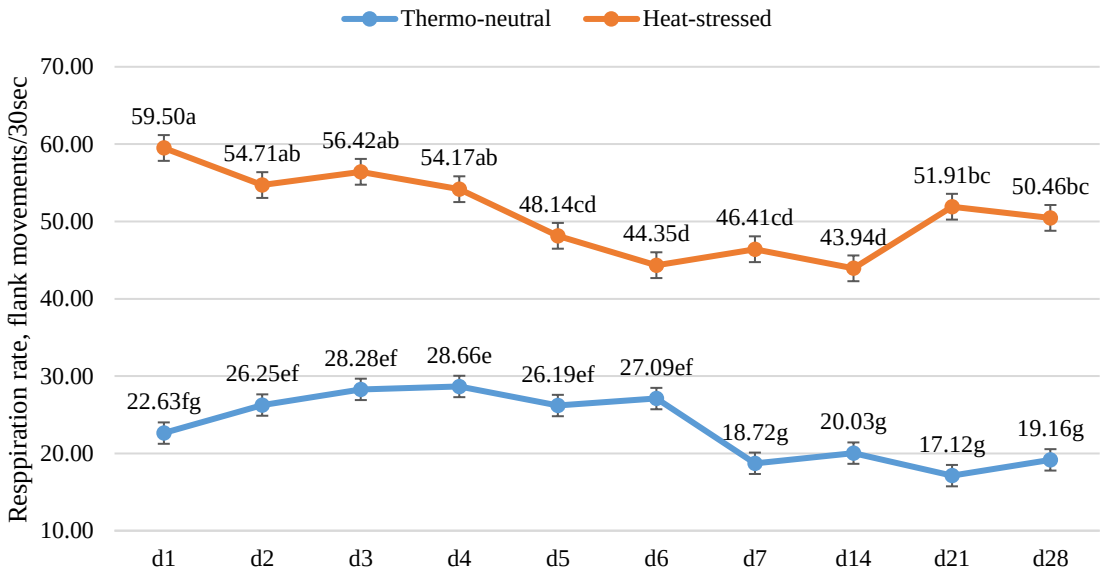



**Figure 1.** Temperature means of thermo-neutral and heat stressed environments from d 1 to 28 in intervals of 10 min at 2400, 0200, 0400, 0600, 0800, 1000, 1200, 1400, 1600, 1800, 2000, and 2200h. Values were recorded by temperature data recorders. **A.** Temperature means for Exp. 1. **B.** Temperature means for Exp. 2.

**A**




**B**

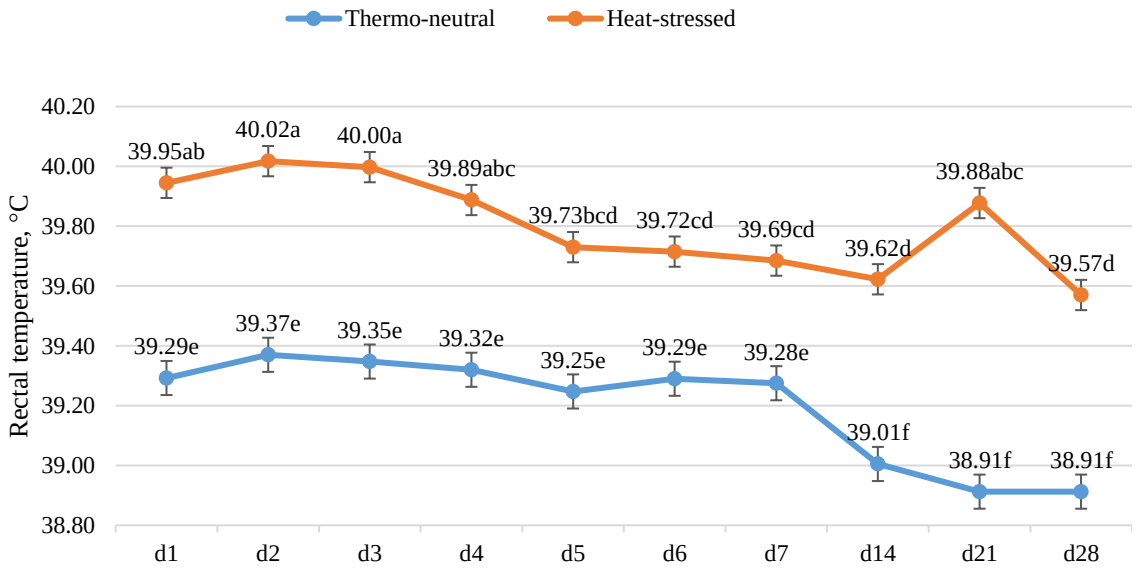



**Figure 2.** Effect of environment on respiration rate and rectal temperature measured on d 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28. Environment x day interaction ($P < 0.001$). Measurements were taken between 1300 and 1600 h (peak of heat stress during the day). Numbers represent least squares means ± SEM of 64 pigs. Means with different superscript are different (a-g) ($P < 0.001$). **A.** Respiration rate in heat stressed pigs differed from d 1 through d 28. Respiration rate on d 0 was not different ($P = 0.128$; heat-stressed environment: 19.64; and thermo-neutral environment: 18.36 respirations per sec). Respiration rate decreased over time within both environments. **B.** Rectal temperatures in heat stressed pigs were greater in comparison with those in the thermo-neutral environment. Rectal temperature on d 0 did not differ between environmental treatments ($P = 0.312$; heat-stressed environment: 39.26 and thermo-neutral environment: 39.40 ºC). Rectal temperature decreased over time for both environments.

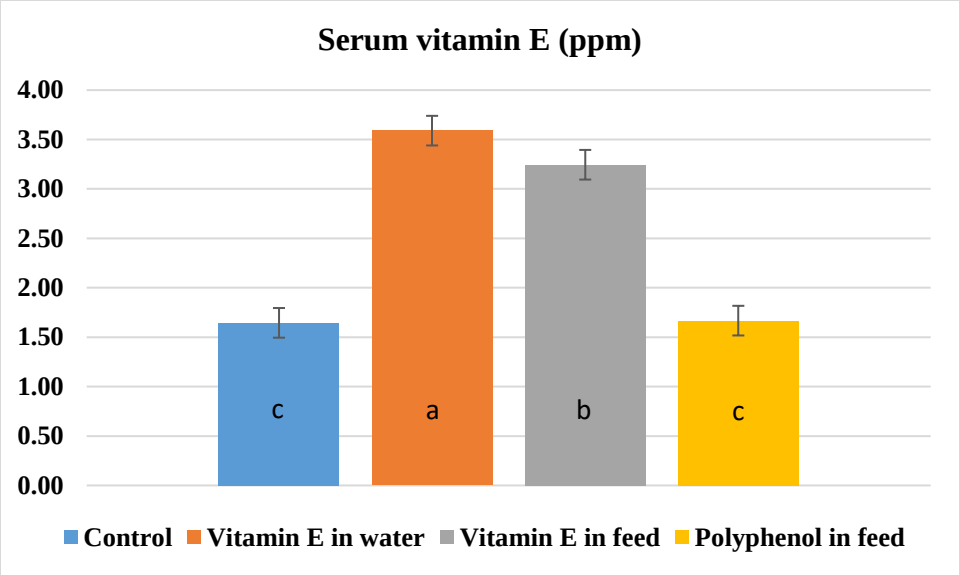



**Figure 3.** Effect of supplementation of vitamin E on vitamin E concentrations in serum in pigs ($P < 0.05$). Serum vitamin E was greater when vitamin E was supplemented in water than when vitamin E was supplemented in feed ($P = 0.002$) with no effects due to polyphenol supplementation ($P \geq 0.05$).

# CHAPTER III:

## Impact of dietary peroxidized lipids and antioxidant supplementation in feed on growth performance, oxidative stress and antioxidant status of newly weaned piglets

**ABSTRACT:** The objective of this study was to evaluate the use of dietary vitamin E and polyphenols on growth and oxidative status of weaned piglets fed peroxidized lipids. Pigs (*n*=192; 21-d of age; 6.62 ± 1.04 kg BW) were assigned within sex and weight blocks to a 2 x 3 factorial arrangement using 48 pens (4 pigs/pen; 8 replications/treatment). Factors consisted of lipid peroxidation (6% human-grade soybean oil or 6% peroxidized soybean oil), and antioxidant supplementation (control diet containing 25 IU/kg dl-α-tocopheryl-acetate; control with 200 IU/kg additional dl-α-tocopheryl-acetate; or control with 400 mg/kg polyphenols). Peroxidation was accomplished by heating control oil at 80°C with constant air flow at 50 L/min for 12 d (initial peroxide, anisidine value, hexanal, and 2,4-decadienal concentrations were 4.1 vs. 141.6 meq/kg, 1.7 vs. 106, <5 vs. 99 mg/kg, and 8 vs. 720 mg/kg for control and peroxidized oil, respectively). Diets were fed in 2 phases (14 and 21 days). Overall, peroxidized lipids decreased ($P < 0.001$) BW (23.16 vs. 18.74 kg), ADG (473 vs. 346 g/day), ADFI (658 vs. 535 g/day) and G:F (719 vs. 647 g/kg). Lipid peroxidation decreased serum vitamin E ($P < 0.001$) and this decrease was larger on d 35 (1.82 vs. 0.81 mg/kg) than d 14 (1.95 vs. 1.38 mg/kg). Supplemental vitamin E, but not polyphenols, increased ($P \leq 0.002$) serum vitamin E by 84 and 22%, for control and peroxidized diets, respectively (interaction, $P = 0.001$). Serum malondialdehyde decreased ($P < 0.001$) with peroxidation on d 14, but not d 35 and protein carbonyl increased ($P < 0.001$) with peroxidation on d 35, but not d 14. Serum 8-hydroxydeoxyguanosine was not affected ($P > 0.05$). Total antioxidant capacity decreased with peroxidation ($P < 0.001$) and increased with vitamin E ($P = 0.065$) and polyphenols ($P = 0.046$) in the control oil diet only. Serum cytokine concentrations of interferon-γ, interleukin (IL)-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, TNF-α, were not impacted by feeding peroxidized lipids or antioxidant supplementation ($P>0.05$). Peroxidized

lipids reduced growth performance, which could not be corrected with supplemental vitamin E or polyphenols in the diet.

**Key words:** immune status, oxidative stress, polyphenols, piglets, vitamin E

## Introduction

Swine diets often include fats or oils to increase the energy density of the diet. Fats and oils are very dense energy sources with a low cost per energy unit provided (NRC, 2012). Lipid peroxidation is formed by oxygen attack on unsaturated fatty acids and with increased high temperature exposure (Kerr *et al*., 2015; Choe and Min, 2007). Unsaturated lipids are more susceptible to peroxidation than saturated fatty acids (Kerr *et al*., 2015). Thus, vegetable oils are more prone to peroxidation than more saturated fats, such as choice white grease and tallow (Choe and Min, 2007).

Fast food restaurant chains use a high amount of vegetable oils to prepare fast food including fries. This cooking process at high temperatures produces high amounts of peroxidized oils (Sebastian *et al*., 2014). Commonly, spent cooking oils are recycled into lipid sources for use in animal feed (Panadare and Rathod, 2015).

Studies evaluating the impact of peroxidized oils in livestock have shown variable results, including no effects on growth performance of rabbits (Blas *et al*., 2010), a reduction in feed intake in sheep (Peixoto *et al*., 2017), and pigs and broilers (Shurson *et al*., 2015), a reduction in growth of pigs (Boler *et al*., 2012; Hanson et al., 2016, Liu *et al*., 2014, Rosero *et al*., 2015 and Lindblom *et al*., 2018b), in broilers (Anjum *et al*., 2002; Shurson *et al*., 2015 and Lindblom *et al*., 2019), and rats (Yen *et al*., 2010), increased morbidity and mortality in pigs (Chang *et al*., 2019), reduced oxidative stability of pork (Dilger *et al*., 2018), no oxidative effects in sheep meat (Capelari *et al*., 2016), alteration of the nutritional quality of chicken and rabbit meat (Tres *et al*., 2013) and

compromised oxidative status in animals (Anjum *et al*., 2002; Yen *et al*., 2010; Sebastian *et al*., 2014, and Lindblom *et al*., 2018a, 2019) causing degradation of cellular components such as protein, DNA and lipids (Lindblom *et al*., 2018a, 2019).

The role of natural antioxidants in the body is to delay or prevent oxidation, and blocking and capturing formed radicals in cells (Pisoschi and Pop, 2015). Vitamin E is a fat-soluble vitamin and natural antioxidant (Sies *et al*., 1992 and Lúcio *et al*., 2009). Likewise, plant polyphenols are antioxidants with high potential against oxidation (Zhang and Tsao, 2016). Several studies reported positive effects of vitamin E or plant polyphenols on meat oxidation in broilers (Goñi *et al*., 2007; Brenes *et al*., 2008), meat quality in finishing pigs (Boler *et al*., 2012), and antioxidant activity in piglets (Luehring *et al*., 2011; Gerasopoulos *et al*., 2015). Moreover, the supplementation of vitamin E and synthetic antioxidant blends or synthetic antioxidants to diets containing peroxidized vegetable oil for weaned piglets or finishing pigs improved growth and reduced lipid and protein oxidation (Lu *et al*., 2014 and Boler *et al*., 2012, respectively). Therefore, the present study was conducted to investigate the potential antioxidant effects of the vitamin E or plant polyphenols when supplemented in diets containing peroxidized oil on growth performance, immune and oxidative status, and the antioxidant activity of weaned pigs.

## Materials and methods

The experimental protocol and procedures were reviewed and approved by the Institutional Animal Care and Use Committee of North Carolina State University (Raleigh, NC, US) (IACUC 18-091-A).

### *Animals and experimental design*

This study was conducted at the Swine Educational Unit (Raleigh, NC). A total of 192 3-week old crossbred pigs (96 gilts and 96 barrows) with an initial body weight of 6.62 ± 1.04 kg

were used. At weaning, piglets were blocked by initial body weight and sex and randomly assigned within blocks to a 2 x 3 factorial arrangement using an experimental allotment program (Kim and Lindemann, 2007). The dietary treatments consisted of the inclusion of 2 types of oils (6% human-grade soybean oil (control oil) or 6% peroxidized soybean oil (peroxidized oil) and 3 antioxidant supplementations: 1) Control diet (25 IU/kg dl-α-tocopheryl acetate) (CON); 2) Control + 200 IU/kg of vitamin E (dl-α-tocopheryl acetate) (Rovimix®, DSM, Heerlen, The Netherlands) (VITE); or control + 400 mg/kg of blend of plant-based polyphenols (POL). Each dietary treatment was randomly assigned to pens of 4 pigs per pen within each block.

The peroxidized oil was made using 163 kg of human-grade soybean oil (Kirkland, Washington, WA), equally divided into two metal barrels. A heater was placed in each barrel and was set to maintain a constant temperature of 80°C for 12 days. In addition, a PVC pipe with 1 mm holes was submerged in each barrel to continuously bubble air through the oil at a constant flow rate of 50 L/min for 12 days.

The dietary treatment feeds were manufactured at the North Carolina State University Feed Mill Educational Unit (Raleigh, NC). Diets were based on corn-soybean meal and were formulated to meet or exceed all nutrient requirements for piglets as suggested by the NRC (2012). At the time of mixing, 0.1% of liquid antioxidant Rendox® (Kemin industries, Inc. Des Moines, IA) containing tertiary butyl hydroquinone (TBHQ) was added to the control oil and the peroxidized oil prior to mixing of feed to avoid further oil peroxidation. A basal mix containing all ingredients, except the oils and supplements was created and divided into 6 batches. Final treatment diets were then created by mixing control oil or peroxidized oil with the basal mix and within these, no supplement, vitamin E, or polyphenols were added at the appropriate concentrations. Diets were fed in 2 phases, with phase 1 being fed immediately after weaning for 14 days (Table 1) and phase

2 being fed the next 21 days (Table 2). All piglets were provided *ad libitum* access to feed and drinking water.

Pigs were allotted into two identical temperature-controlled rooms (Aerotech® AeroSpeed 1.2, Pittsburgh, PA) containing 24 pens each. Pigs were housed at 4 pigs per pen (2 gilts and 2 barrows). The size of each pen was 0.91 m x 1.52 m and pens contained a stainless-steel pig feeder type single-sided-2 spaces (Staco® Inc, Schaeffer town, PA) and two stainless steel water nipples for weaned piglets (Hog slat®, Newton Grove, NC). Environmental temperatures and ventilation were checked every morning. Temperatures were set at 32°C for the first week and then reduced by 2°C throughout each week until the temperature reached 24°C.

***Growth performance***

Body weight (BW) was measured on d 0, 7, 14, 21, 28 and 35 to calculate average daily gain (ADG). Daily feed intake was measured from the difference between daily feed additions and feed remaining at the end of the week or phase and divided by 7 days or days in the phase. Feed efficiency (G: F) was calculated by dividing ADG by ADFI weekly and for each phase.

***Serum sample collection***

Blood samples from one randomly selected pig per pen were collected by venipuncture (jugular vein) using 20-gauge x 3.8 cm multiple use drawing needles (Vacuette, Greiner bio-one, Kremsmunster, Austria) on d 14 and d 35 (at approximately 0900). Blood was collected into 10 mL-vacuum tubes (BD Vacutainer Serum, Franklin Lakes, NJ). Blood was centrifuged at 4,267 x g for 10 min at 4°C using a refrigerated centrifuge (Centra GP8R, Thermo IEC, MA) and serum was collected. Serum was aliquoted into 6 tubes of 2 mL capacity (Biotix®, Neptune, 3472.X, Mesa Rim, San Diego, CA) and stored at -80°C for further analysis.

***Chemical Analysis***

Proximate analysis of the diets was conducted by the Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO) using AOAC Official methods (2006). Diets were analyzed for moisture (Method 934. 01), crude protein (Method 990. 03), crude fat (Method 920. 39 (A)), crude fiber (Method 978. 10), ash (Method 942.05), Neutral Detergent Fiber (JAOAC 56, 1352-1356, 1973) Acid Detergent Fiber (Method 973. 18 (A-D)), Calcium (Method 985.01 (A, B, D)) and Phosphorus (Method 966. 01).

Oil samples from control and peroxidized oil were collected and analyzed by New Jersey Feed Laboratory Inc., (Trenton, NJ) using AOAC (1990) and AOCS (1998) procedures and by Kemin Industries, Inc. (Des Moines, IA).

***Vitamin E concentration in serum***

Vitamin E analysis of serum samples collected on d 14 and d 35 was conducted by the Veterinary Diagnostic Laboratory at Iowa State University (Ames, IA). Samples were analyzed using high performance liquid chromatography.

***Immune status in serum***

Serum samples for both d 14 and d 35 were submitted to Eve Technologies Corporation (Calgary, Canada) for analysis of pro- and anti-inflammatory cytokines using Luminex xMAP Multi-plex technique (multiplex technology). Results of cytokines from IFN-$\gamma$, IL-1$\alpha$, IL-1$\beta$, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and TNF-$\alpha$ were reported and expressed in pg/mL of serum.

***Oxidative status in serum***

Malondialdehyde (MDA) was measured in serum samples collected on d 14 and d 35 using Oxiselect™ TBARS assay kit (MDA Quantitation; Cell BioLabs, Inc., San Diego, CA) catalog

number STA330. Absorbance was measured at 532 nm on a microplate reader (Bio Tek Instruments®, Synergy HT, Winooski, VT) and using a software program (KC4™, Bio Tek Instruments®, Winooski, VT). Results from MDA for serum samples were expressed in µM. Intra assay CV was 9%.

8-hydroxydeoxyguanosine (8-OHdG) was measured in serum samples on d 14 and d 35 using Oxiselect™ Oxidative DNA Damage ELISA kit (8-OHdG Quantitation; Cell BioLabs, Inc., San Diego, CA) catalog number STA320. Absorbance was measured at 450 nm on a microplate reader (Bio Tek Instruments®, Synergy HT, Winooski, VT) and using a software program (KC4™, Bio Tek Instruments®, Winooski, VT). Results were expressed in ng/mL. Intra-and inter-assay CV were 4 and 3%, respectively.

Protein carbonyl was measured in serum samples using the protein carbonyl colorimetric assay kit (Cayman Chemical®, Ann Arbor, MI). Protein carbonyl was expressed based on protein concentration, which was determined using the BCA Protein Assay kit (Fisher Scientific®, Hampton, NH). Absorbance was measured at 360 nm on a microplate reader (Bio Tek Instruments®, Synergy HT, Winooski, VT) and using a software program (KC4™, Bio Tek Instruments®, Winooski, VT). Results were expressed in pmol carbonyl/mg of protein. Intra-and inter-essay CV were 5 and 3%, respectively.

***Antioxidant activity***

Total antioxidant capacity (TAC) on was measured in serum samples using Oxiselect™ Total Antioxidant Capacity (TAC) assay kit protocol (Cell BioLabs, Inc., San Diego, CA) catalog number STA360. Absorbance was measured at 490 nm on a microplate reader (Bio Tek Instruments®, Synergy HT, Winooski, VT) and using a software KC4™ program (Bio Tek Instruments®, Winooski, VT). Results were expressed as µM Copper Reducing Equivalents

(CRE). CRE sample values are proportional to the sample's total antioxidant capacity. Intra-and inter-essay CV were 2.1 and 3.5%, respectively.

***Statistical analysis***

The data for growth performance and serum measurements were analyzed using the Proc MIXED procedure of SAS (v.9.4, SAS Institute. Inc., Cary, NC). For growth performance measurements, pen was used as the experimental unit and weight block was the random effect. The statistical model included block, type of oil, supplementation and the interaction between oil type and supplementation. For serum measurements, individual pig was considered the experimental unit. The MIXED Procedure was used. The statistical model included block, type of oil, supplementation, day of collection and the appropriate interactions. Statistical significances were considered at $P < 0.05$ and tendencies at $0.05 \leq P \leq 0.10$.

**Results**

***Chemical analysis of peroxidized soybean oil***

Results of chemical analysis of peroxidized and control soybean oil are shown in Table 3. Initial peroxidized value for the peroxidized oil was higher than the control oil (141.6 vs. 4.1mEq/kg fat). Likewise, 4 hr and 20 hr AOM value in peroxidized oil were increased relative to control oil (3.6 vs 158 and 4.6 vs. 41.1, respectively). Anisidine value, hexanal, and 2,4-decadienal levels were highly increased in the peroxidized oil (1.7 vs. 106, <5 vs. 99 ppm and 8 vs. 720 ppm, respectively).

***Growth performance***

Two pigs, one from a pen of peroxidized oil treatment + vitamin E, and one from a pen of control oil + polyphenols treatment, were removed from the study due to significant losses in BW.

No interactions between peroxidation and supplementation were observed for growth performance ($P > 0.05$). Peroxidation and antioxidant supplementation ($P \geq 0.05$) did not impact BW on d 7 and 14 (Table 4). However, peroxidation reduced BW ($P < 0.001$) on d 21 (-7.8 %), d 28 (-13.8 %) and d 35 (-19.1 %). ADG was reduced ($P < 0.05$) by peroxidation during week 2 (-7.6 %), 3 (-27.3 %), 4 (-32.1 %), 5 (-35.3 %), phase 2 (-32.4 %) and overall (-26.9 %). In addition, ADG tended to be increased by supplementation of antioxidants ($P = 0.063$). ADFI tended to be reduced by peroxidation for week 2 ($P = 0.093$; -5.6 %), and was reduced for week 3 ($P < 0.001$; -13.1 %), 4 ($P < 0.001$; -22.8 %), 5 ($P < 0.001$; -23.1 %), phase 2 ($P < 0.001$; -22.2 %), and overall ($P < 0.001$; -18.7 %). Peroxidation reduced ($P < 0.05$) G:F for week 3 (-15.5%), 4 (-12.4 %), 5 (-15.9 %), phase 2 (-13.5 %) and overall (-10.1 %). In addition, G:F was reduced (-10%) by vitamin E supplementation for week 3 ($P = 0.019$) and tended to be improved (+3%) by polyphenol supplementation for the overall period ($P = 0.074$). During the first week of the study, peroxidation reduced (-8.5 %) G:F in pigs fed vitamin E and increased (+14 %) in pigs fed polyphenol treatment (interaction, $P = 0.084$).

***Vitamin E in serum***

Peroxidation decreased serum vitamin E concentrations by 41.8% ($P < 0.001$; Table 5) and this reduction in vitamin E concentration was greater on d 35 compared to d 14 (day x peroxidation interaction, $P < 0.001$). Supplementation of vitamin E increased serum vitamin E concentrations ($P < 0.001$) compared to control and polyphenols treatments (1.98 vs. 1.25 and 1.26 ppm, respectively) and this increase was greater for the control diet compared to the diets with peroxidized oil (interaction, $P < 0.001$). The three-factor interaction between peroxidation, supplementation and day of measurement was not significant ($P = 0.101$).

***Oxidative status in serum***

Peroxidation, supplementation and day of sampling did not significantly affect serum 8-OHdG ($P \geq 0.05$; Table 6). Protein carbonyl was increased (+24%) by peroxidation ($P < 0.001$) and was increased (42.5%) on d 35 when compared with d 14 of sample collection ($P < 0.001$). Protein carbonyl in serum increased when peroxidized oil was fed on d 35 (interaction, $P < 0.001$) but not on d 14. Peroxidation increased serum protein carbonyl (2.9 vs. 2.1 pmol/mg protein) for the polyphenol treatment (interaction, $P < 0.05$) but not for the control or vitamin E treatments. The peroxidized oil diet decreased MDA in serum ($P < 0.001$); likewise, on d 35 serum MDA increased when compared with d 14 ($P < 0.05$). On d 14, peroxidized oil decreased MDA when compared with control oil (8.5 vs. 12.7) (interaction; $P < 0.001$) but no effects were observed on d 35 with peroxidation.

***Antioxidant activity***

Serum TAC was decreased (-10.6%) by peroxidation ($P < 0.001$). Additionally, TAC in serum increased on d 35 when compared on d 14 (+18%; $P < 0.001$; Table 6). TAC was reduced by peroxidation and vitamin E (-16.6%) and for polyphenols supplementation (-21.5%) (interaction, $P = 0.045$). In addition, TAC was increased on d 35 by supplementation of vitamin E (+15%) and polyphenols (+27%) when compared to d 14 (interaction, $P = 0.025$).

***Immune status in serum***

Peroxidation did not impact ($P \geq 0.05$) serum concentrations of pro- and anti-inflammatories cytokines IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, and TNF-α. (Table 7). Cytokines IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-10, IL-12, and IL-18 were reduced and IFN-γ increased on d 35 compared to d 14 ($P < 0.05$). Dietary vitamin E and polyphenols reduced ($P < 0.10$) IL-1 β, IL-1ra, IL-2 and IL-4 when compared with the control

treatment. Additionally, on d 14 peroxidation reduced IL-1α, IL-β, IL-1ra, IL-2, IL-4, IL-6, IL-10, IL-12, and IL-18, but they were increased by peroxidation on d 35 (interaction, $P < 0.05$).

**Discussion**

Feeding peroxidized oil to pigs negatively affects growth performance (DeRouchey *et al*., 2004, Rosero *et al*., 2015 and Hung *et al*., 2017) and this decrease appears to be directly related to the extent of peroxidation. In the present study, the pigs had reduced growth performance from d 14 through 35 (phase 2), but not for the initial 14 days (phase 1). The first phase diet was complex in composition, containing whey permeate, spray-dried plasma protein, and highly digestible animal-based proteins ingredients, which are highly palatable, especially for the first 2 weeks after weaning (van Dijk *et al*., 2001 and Sugiharto *et al*., 2015). Perhaps these ingredients allowed pigs to maintain feed intake in the presence of negative odor and taste associated with aldehydes in peroxidized oil that are responsible for rancid odor and flavor (Tompkins and Perkins, 1999). On the other hand, there may be a threshold beyond which the impact of peroxidized lipids manifests itself. The fact that detrimental effects of dietary peroxidized soybean oil is expressed more in the long term is supported by Anjum *et al*. (2010) and Lu *et al*. (2014) in broilers and in pigs, respectively. DeRouchey *et al*. (2004), Liu *et al*. (2014) and Rosero *et al*. (2015) reported reductions in ADFI in pigs when using peroxidized lipids and these effects were dose-dependent. The reduction in growth performance is due in part to the negative impact in the intestine caused by dietary peroxidized oil fed to piglets (Rosero *et al*., 2015). The addition of peroxidized oil in the diet for pigs causes oxidative stress (Boler *et al*., 2012 and Hanson *et al*., 2016), but the supplementation of dietary vitamin E (da Rocha *et al*., 2012) and polyphenols (Gessner *et al*., 2017) could ameliorate the negative effects caused by oxidation (Boler *et al*., 2012; Hung *et al*., 2017). In the present study, the supplementation of vitamin E reduced G:F on d 21. These results

agree partially with those published by Boler *et al*. (2012), who found no effects of supplementation with tert-butylhydroquinone and ethoxyquin blend antioxidant to a diet containing peroxidized corn oil in finishing pigs. Rooke *et al*. (2004) reported that an antioxidant blend or antioxidant blend plus vitamin E improved growth performance when feeding 5% of dietary peroxidized oil and showed regeneration of vitamin E by vitamin C and glutathione peroxidase. Peroxidation products could reduce the function of fat-soluble vitamins (Lu *et al*., 2014).

Several authors reported that peroxidized oil reduced serum concentration of vitamin E due to catabolism of the vitamin E during oxidative stress (Boler *et al*., 2012; Shurson *et al*., 2015; Buchet *et al*., 2017; Hung *et al*., 2017, and Chang *et al*., 2019). This fact agrees with the results found in the present study. Serum vitamin E was reduced with peroxidation, showing the ability of vitamin E to react against oxidation. Vitamin E is a part of all antioxidant actions present in plasma, body fluids, and cell membranes. TAC measures all antioxidant actions; therefore, vitamin E is positive correlated and partially contributed to TAC results (Sies, 2007). The concentration of serum vitamin E in peroxidized oil treatment with polyphenols was lower than control oil, indicating that polyphenols were not able to regenerate the vitamin E once it was oxidized. This could be related to low absorption rate of dietary polyphenols (Gessner *et al*., 2013) or the type of polyphenols used. Peroxidized lipids damage proteins leading to malfunction and alteration in their structure increasing protein carbonyls. Protein carbonyl is a biomarker with high stability and it is formed during the early period of oxidative stress (Mateos and Bravo, 2007). In the present study, protein carbonyl in serum was increased by peroxidation, particularly on d 35, but supplementation with antioxidants did not ameliorate the effect. Likewise, Lindblom *et al*. (2018a) and Lu *et al*. (2014) reported increased protein carbonyl in serum of pigs fed peroxidized oil. Boler *et al*. (2012)

did not find differences in protein carbonyls in plasma when using a synthetic antioxidant blend supplemented to diets containing peroxidized corn oil when compared with peroxidized corn oil diet without antioxidant in pigs. But a study conducted by Lu *et al*. (2014) showed significant decreases in plasma carbonyls on d 55 in growing pigs fed peroxidized lipids when using dietary vitamin E and a synthetic antioxidant blend, but this was not the case on d 118, indicating that long-term supplementation of peroxidized oil may overwhelm the antioxidant system resulting in increased markers of oxidative stress.

8-OHdG is an oxidized nucleoside of DNA detected during DNA oxidation (Mateos and Bravo, 2007). Comparing with other oxidative status markers tested in this study, serum 8-OHdG was not affected by peroxidation and antioxidant supplementation. Likewise, Lindblom *et al*. (2018a) and Chang *et al*. (2019) did not report differences in DNA damage using various levels of peroxidation of supplemental oil. The site of DNA damage occurs in nuclear and mitochondrial DNA in tissue and in DNA of lymphocytes (Duthie *et al*., 1996 and Wu *et al*., 2004). Lymphokines which are derived from lymphocyte stimulation and synthesized by lymphocytes clones, include IL-1, IL2, IL-3, IL-4, IL-5, IL-6, TNF-α and IFN-γ (Hamblin, 1988). Lymphokines contribute to cellular DNA damage. The cytokines evaluated in this experiment were not affected by peroxidation, which is similar to the serum 8-OHdG results.

MDA is a three-carbon cytotoxic molecule, produced by peroxidation of polyunsaturated fatty acids (Mateos and Bravo, 2007). The serum concentration of MDA, as a marker of lipid peroxidation status, increased due to the consumption of peroxidized oil (Anjum *et al*., 2002, Yen *et al*., 2010, Boler *et al*., 2012, and Lu *et al*., 2014). Likewise, Hung *et al*. (2017) compiled 65 observations of MDA in studies from poultry and swine finding 6% of significant increases and 20% of non-significant differences and the rest of the observational data were not reported.

Therefore, some evidence was found that MDA increased when using peroxidized oil in diets fed to pigs and poultry. In the present study, MDA decreased with peroxidized oil when measured on d 14 and 35, and MDA levels were higher on d 35 than d 14. Contrarily, Chang *et al*. (2019) did not report differences in serum MDA due to lipid peroxidation in weaned piglets.

TAC is defined as the sum of all corporal antioxidant actions in the body including the major small-molecule contributors of TAC (urate, ascorbate, and tocopherol) (Sies, 2007). In the present study, TAC was reduced by peroxidation. Similarly, serum vitamin E was reduced due to peroxidation. These results agree with those presented by Yen *et al*. (2010) in rats which consumed a diet with 15% of deep-frying oils, showing lower serum vitamin E levels. Values of TAC were higher on d 35 compared to d 14, indicating increased antioxidant capacity as pigs grew and a reflection of increased feed consumption. In addition, it was observed that supplementation of vitamin E and polyphenols to pigs fed peroxidized oil reduced TAC, suggesting that antioxidants reacted against oxidation. Similar results were found by Yen *et al*. (2010) using peroxidized soybean oil in rats. Interestingly, on d 35, higher TAC levels were found for the polyphenols treatment than the control and dietary vitamin E treatment, suggesting antioxidant efficiency of polyphenols increased with time. Contrarily, Lipinski *et al*. (2017) concluded that polyphenols are deposited in the organs which participate in their metabolism, with plasma concentrations remaining lower during long periods or high doses in pigs. Moreover, no improvements in TAC were reported by Gessner *et al*. (2013), who used 1% of dietary polyphenols in diets of piglets. Also, during metabolism of polyphenols (flavonoids) uric acid is produced as part of TAC (Sies, 2007). Thus, high levels of TAC resulting from POL treatments may be due to metabolic products derived from the commercial polyphenols used in this study.

Peroxidation caused lipid oxidation and inflammation in intestinal cells, producing an increase in cytokines levels (Lingaraju *et al*., 2015). In the present study, peroxidation did not affect serum concentrations of pro- and anti-inflammatory cytokines. This suggests that the levels of peroxidation may not have been enough to induce inflammation in pigs regardless of the negative impact produced on growth performance. Also, Rosero *et al*. (2015) did not find differences on the pro-inflammatory cytokine TNF-α when peroxidized oils were added in the diet for piglets. The high levels of the majority of cytokines on d 14 compared to d 35 may be due to the early weaning effects on pigs (Moeser *et al*. 2017). Interestingly, cytokines increased by peroxidation on d 35 and can be related to the reduction in growth performance at the end of the study.

In conclusion, the addition of peroxidized soybean oil negatively affected growth performance from d 21 to 28, d 28 to 35, during phase 2, and overall. Moreover, peroxidation negatively affected serum vitamin E concentrations, increased oxidative stress status and serum cytokines concentrations, but not DNA oxidation. Even though lipid peroxidation measured by MDA was decreased by peroxidized lipids, the total antioxidant capacity was positive affected by vitamin E and polyphenols. No improvements in growth performance and oxidative status with vitamin E and polyphenol supplementations were detected, with the exception of some cytokine concentrations.

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**References**

Anjum, M. I., Alam, M. Z., & Mirza, I. H. (2002). Effect of non-oxidized and oxidized soybean oil supplemented with two levels of antioxidant on broiler performance. *Asian-Australasian Journal of Animal Sciences, 15*(5), 713-720. doi:10.5713/ajas.2002.713

AOAC. Official methods of analysis. 15th ed. Arlington (VA): AOAC; 1990

AOCS. Official methods and recommended practices of the American Oil Chemists Society. 5th ed. Champaign (IL): AOCS; 1998

AOAC. Official Methods of Analysis. 18th Edition, Association of Official Analytical Chemists, Gaithersburgs, MD; 2006

Blas, E., Cervera, C., Rodenas, L., Martínez, E., & Pascual, J. J. (2010). The use of recycled oils from the food industry in growing rabbit feeds in substitution of fresh oil does not affect performance. *Animal Feed Science and Technology, 161*(1), 67-74. doi:10.1016/j.anifeedsci.2010.07.011

Boler, D. D., Fernández-Dueñas, D. M., Kutzler, L. W., Zhao, J., Harrell, R. J., Campion, D. R., McKeith, F. K., Killeger, J., & Dilger, A. C. (2012). Effects of oxidized corn oil and a synthetic antioxidant blend on performance, oxidative status of tissues, and fresh meat quality in finishing barrows. *Journal of Animal Science, 90*(13), 5159-5169. doi:10.2527/jas.2012-5266

Brenes, A., Viveros, A., Goñi, I., Centeno, C., Sáyago-Ayerdy, S. G., Arija, I., & Saura-Calixto, F. (2008). Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poultry Science, 87*(2), 307-316. doi:10.3382/ps.2007-00297

Buchet, A., Belloc, C., Leblanc-Maridor, M. & Merlot, E. (2017). Effects of age and weaning conditions on blood indicators of oxidative status in pigs. *PLoS One, 12*(5), e0178487. doi:10.1371/journal.pone.0178487

Capelari, M., Peixoto, E. L. T., Moura, E. S., Ribeiro, E. L. A., & Mizubuti, I. Y. (2016). Meat quality of lambs fed diets containing different levels of residual frying oil. *Journal of Animal Science, 94*, 427-428. doi.org/10.2527/jam2016-0889

Chang, P. L., Boyd, R. D., Zier-Rush, C., Rosero, D. S., & van Heugten, E. (2019). Lipid peroxidation impairs growth and viability of nursery pigs reared under commercial conditions. *Journal of Animal Science,* doi:10.1093/jas/skz183

Choe, E., & Min, D. B. (2007). Chemistry of Deep-Fat frying oils. *Journal of Food Science, 72*(5), R86. doi:10.1111/j.1750-3841.2007.00352.x

da Rocha, C., Maiorka, A., de Paula Valle, F. L, Gonsales Schramm, V., Angeli, A. L., & Fischer da Silva, A. V. (2012). The effect of soybean oil quality and vitamin E supplementation on turkey diet nutrition. *Journal of Applied Poultry Research, 21*(2), 318-324. doi:10.3382/japr.2011-00407

DeRouchey, J. M., Hancock, J. D., Hines, R. H., Maloney, C. A., Lee, D. J., Cao, H., Dean, D. W., Park, J. S. (2004). Effects of rancidity and free fatty acids in choice white grease on growth performance and nutrient digestibility in weanling pigs. *Journal of Animal Science, 82*(10), 2937-2944. doi:10.2527/2004.82102937x

Dilger, A.C., Overholt, M.F., Boler, D. D., Kerr, B. J. 274 Impact of Feeding Oxidized Oils to Induce Oxidative Stress on the Quality of Pork., *Journal of Animal Science,* Volume 96, Issue suppl_2, April 2018, Page 147, doi.org/10.1093/jas/sky073.271

Duthie, S. J., Ma, A., Ross, M. A., & Collins, A. R. (1996). Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Research, 56*(6), 1291-1295.

Gerasopoulos, K., Stagos, D., Petrotos, K., Kokkas, S., Kantas, D., Goulas, P., & Kouretas, D. (2015). Feed supplemented with polyphenolic byproduct from olive mill wastewater processing improves the redox status in blood and tissues of piglets. doi://doi.org/10.1016/j.fct.2015.11.007

Gessner, D. K., Fiesel, A., Most, E., Dinges, J., Wen, G., Ringseis, R., & Eder, K. (2013). Supplementation of a grape seed and grape marc meal extract decreases activities of the oxidative stress-responsive transcription factors NF-κB and Nrf2 in the duodenal mucosa of pigs. *Acta Veterinaria Scandinavica, 55*(1), 18. doi:10.1186/1751-0147-55-18

Gessner, D. K., Ringseis, R., & Eder, K. (2017). Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. *Journal of Animal Physiology and Animal Nutrition, 101*(4), 605-628. doi:10.1111/jpn.12579

Goñi, I., Brenes, A., Centeno, C., Viveros, A., Saura-Calixto, F., Rebolé, A., Arija, I., & Estevez, R. (2007). Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens. *Poultry Science, 86*(3), 508-516. doi:10.1093/ps/86.3.508

Hamblin A. S. (1988). Lymphokines and interleukins. *Immunology, 64*(Suppl 1), 39–41.

Hanson, A. R., Urriola, P. E., Wang, L., Johnston, L. J., Chen, C., & Shurson, G. C. (2016). Dietary peroxidized maize oil affects the growth performance and antioxidant status of nursery pigs. *Animal Feed Science and Technology, 216,* 251-261. doi:10.1016/j.anifeedsci.2016.03.027

Hung, Y. T., Hanson, A. R., Shurson, G. C., & Urriola, P. E. (2017). Peroxidized lipids reduce growth performance of poultry and swine: A meta-analysis. *Animal Feed Science and Technology, 231,* 47-58. doi:10.1016/j.anifeedsci.2017.06.013

Kerr, B. J., Kellner, T. A., & Shurson, G. C. (2015). Characteristics of lipids and their feeding value in swine diets. *Journal of Animal Science and Biotechnology, 6*(1), 30. doi:10.1186/s40104-015-0028-x

Kim, B.G., and M.D. Lindemann. 2007. A spreadsheet method for experimental animal allotment. J. Anim. Sci.85 (Suppl. 2):112.

Lindblom, S. C., Gabler, N. K., Bobeck, E. A., & Kerr, B. J. (2019). Oil source and peroxidation status interactively affect growth performance and oxidative status in broilers from 4 to 25 d of age. *Poultry Science, 98*(4), 1749. doi: 10.3382/ps/pey547

Lindblom, S. C., N. K. Gabler, R. N. Dilger, Z. F. Olson, C. L. Loving, and B. J. Kerr. (2018a). Influence of feeding thermally peroxidized soybean oil on oxidative status in growing pigs. *Journal of Animal Science*, 96(2),545–557. doi:10.1093/jas/sky005

Lindblom, S. C., Gabler, N. K., & Kerr, B. J. (2018b). Influence of feeding thermally peroxidized soybean oil on growth performance, digestibility, and gut integrity in growing pigs. *Journal of Animal Science, 96*(2), 558-569. doi:10.1093/jas/sky004

Lingaraju, A., Long, T. M., Wang, Y., Austin, J. R., & Turner, J. R. (2015). Conceptual barriers to understanding physical barriers. *Seminars in Cell and Developmental Biology, 42*, 13-21. doi:10.1016/j.semcdb.2015.04.008

Lipiński K., Mazur, M., Antoszkiewicz, Z. & Purwin, C. (2017). Polyphenols in monogastric nutrition – A review. *Annals of Animal Science, 17*(1), 41-58. doi:10.1515/aoas-2016-0042

Liu, P., Chen, C., Kerr, B. J., Weber, T. E., Johnston, L. J., & Shurson, G. C. (2014). Influence of thermally oxidized vegetable oils and animal fats on growth performance, liver gene expression, and liver and serum cholesterol and triglycerides in young pigs. *Journal of Animal Science, 92*(7), 2960-2970. doi:10.2527/jas.2012-5709

Lu, T., Harper, A. F., Zhao, J., Estienne, M. J., & Dalloul, R. A. (2014). Supplementing antioxidants to pigs fed diets high in oxidants: I. effects on growth performance, liver function, and oxidative status. *Journal of Animal Science, 92*(12), 5455-5463. doi:10.2527/jas.2013-7109

Lúcio, M., Nunes, C., Gaspar, D., Ferreira, H., Lima, J., & Reis, S. (2009). Antioxidant activity of vitamin E and trolox: Understanding of the factors that govern lipid peroxidation studies in vitro. *Food Biophysics, 4*(4), 312-320. doi:10.1007/s11483-009-9129-4

Luehring, M., Blank, R., & Wolffram, S. (2011). Vitamin E-sparing and vitamin E-independent antioxidative effects of the flavonol quercetin in growing pigs. *Animal Feed Science and Technology, 169*(3-4), 199-207. doi:10.1016/j.anifeedsci.2011.06.006

Mateos, R., & Bravo, L. (2007). Chromatographic and electrophoretic methods for the analysis of biomarkers of oxidative damage to macromolecules (DNA, lipids, and proteins). *Journal of Separation Science, 30*(2), 175-191. doi:10.1002/jssc.200600314

Moeser, A. J., Pohl, C. S., & Rajput, M. (2017). Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. *Animal Nutrition, 3*(4), 313-321. doi:10.1016/j.aninu.2017.06.003

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Panadare, D., Rathod, V. (2015). Applications of waste cooking oil other than biodiesel: A review. *Iranian Journal of Chemical Engineering (IJChE),* 12(3), 55-76.

Peixoto, E. L. T., Mizubuti, I. Y., Ribeiro, E. L. D., Moura, E. D., Pereira, E. S., do Prado, Odimari, O. P. P., De Carvalho, L. N., & Pires, K. A. (2017). Residual frying oil in the diets of sheep: Intake, digestibility, nitrogen balance and ruminal parameters. *Asian-Australasian Journal of Animal Sciences, 30*(1), 51-56. doi:10.5713/ajas.15.0970

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry, 97*, 55-74. doi:10.1016/j.ejmech.2015.04.040

Rooke, J., Robinson, J., & Arthur, J. (2004). Effects of vitamin E and selenium on the performance and immune status of ewes and lambs. *The Journal of Agricultural Science,142*(3), 253-262. doi:10.1017/S0021859604004368

Rosero, D. S., Odle, J., Moeser, A. J., Boyd, R. D., & van Heugten, E. (2015). Peroxidised dietary lipids impair intestinal function and morphology of the small intestine villi of nursery pigs in a dose-dependent manner. *The British Journal of Nutrition, 114*(12), 1985-1992. doi:10.1017/S000711451500392X

Sebastian, A., Ghazani, S. M., & Marangoni, A. G. (2014). Quality and safety of frying oils used in restaurants. *Food Research International, 64*, 420-423. doi:10.1016/j.foodres.2014.07.033

Shurson, G. C., Kerr, B. J., & Hanson, A. R. (2015). Evaluating the quality of feed fats and oils and their effects on pig growth performance. *Journal of Animal Science and Biotechnology, 6*(1), 10. doi:10.1186/s40104-015-0005-4

Sies, H. (2007). Total antioxidant capacity: Appraisal of a Concept. *The Journal of Nutrition, 137*(6), 1493. doi:10.1093/jn/137.6.1493

Sies, H., Stahl, W., & Sundquist, A. R. (1992). Antioxidant functions of vitamins. *Annals of the New York Academy of Sciences, 669*(1), 7-20. doi:10.1111/j.1749-6632.1992.tb17085.x

Sugiharto, S., Lauridsen, C., & Jensen, B. B. (2015). Gastrointestinal ecosystem and immunological responses in E. coli challenged pigs after weaning fed liquid diets containing whey permeate fermented with different lactic acid bacteria. *Animal Feed Science and Technology, 207*, 278-282. doi:10.1016/j.anifeedsci.2015.06.019

Tompkins, C., & Perkins, E. (1999). The evaluation of frying oils with the p-anisidine value. *Journal of the American Oil Chemists' Society, 76*(8), 945-947. doi:10.1007/s11746-999-0111-6

Tres, A., Bou, R., Guardiola, F., Nuchi, C. D., Magrinyà, N., & Codony, R. (2013). Use of recovered frying oils in chicken and rabbit feeds: Effect on the fatty acid and tocol composition and on the oxidation levels of meat, liver and plasma. *Animal: An International Journal of Animal Bioscience, 7*(3), 505-17. doi.org/10.1017/S1751731112001607

van Dijk, A. J., Everts, H., Nabuurs, M. J. A., Margry, R. J. C. F., & Beynen, A. C. (2001). Growth performance of weanling pigs fed spray-dried animal plasma: A review. *Livestock Production Science, 68*(2), 263-274. doi:10.1016/S0301-6226(00)00229-3

Wu, J. T., Wu, L. L., Chiou, C., & Chang, P. (2004). *Urinary 8-OHdG: A marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics*. Netherlands: Elsevier B.V. doi:10.1016/j.cccn.2003.09.010

Yen, Pei-Ling, M.S. Chen, Bing-Huei, Ph.D. Yang, Feili Lo, Ph.D. Lu, Yi-Fa, Ph.D. (2010). Effects of deep-frying oil on blood pressure and oxidative stress in spontaneously hypertensive and normotensive rats. *Nutrition, 26*(3), 331-336. doi:10.1016/j.nut.2009.04.020

Zhang, H., & Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science, 8*, 33-42. doi:10.1016/j.cofs.2016.02.002

**Table 1.** Composition of the experimental Phase 1diets. As fed basis[1]

| | Treatments | | |
|---|---|---|---|
| Item | **CON** | **VITE** | **POL** |
| **Ingredient, %** | | | |
| Corn, yellow dent | 36.35 | 36.35 | 36.35 |
| Soybean meal, 47.5% CP | 21.00 | 21.00 | 21.00 |
| Whey permeate | 20.00 | 20.00 | 20.00 |
| Poultry byproduct meal | 6.76 | 6.76 | 6.76 |
| Fish meal, menhaden | 4.00 | 4.00 | 4.00 |
| Plasma spray-dried | 4.00 | 4.00 | 4.00 |
| Soybean oil[2] | 6.00 | 6.00 | 6.00 |
| Monocalcium phosphate 21% | 0.35 | 0.35 | 0.35 |
| Limestone | 0.24 | 0.24 | 0.24 |
| Zinc oxide, 72% Zn | 0.26 | 0.26 | 0.26 |
| Copper sulfate, 25.2% Cu | 0.07 | 0.07 | 0.07 |
| L-lysine HCl 78.5% | 0.27 | 0.27 | 0.27 |
| DL-methionine | 0.20 | 0.20 | 0.20 |
| L-threonine | 0.13 | 0.13 | 0.13 |
| L-tryptophan | 0.01 | 0.01 | 0.01 |
| Salt | 0.15 | 0.15 | 0.15 |
| Mineral premix[3] | 0.15 | 0.15 | 0.15 |
| Vitamin premix[4] | 0.05 | 0.05 | 0.05 |
| Vitamin E, IU/kg[5] | - | 200.00 | - |
| Polyphenols, mg/kg | - | - | 400.00 |
| **Calculated Composition** | | | |
| ME, kcal/kg | 3609 | 3609 | 3609 |
| Crude Protein % | 24.20 | 24.20 | 24.20 |
| Lactose % | 16.00 | 16.00 | 16.00 |
| SID Amino acids | | | |
| Lys % | 1.50 | 1.50 | 1.50 |
| Thr % | 0.93 | 0.93 | 0.93 |
| Met % | 0.53 | 0.53 | 0.53 |
| Met+Cys% | 0.87 | 0.87 | 0.87 |
| Trp % | 0.27 | 0.27 | 0.27 |
| Total Lysine % | 1.70 | 1.70 | 1.70 |
| **Analyzed composition[6]** | | | |
| Crude protein, % | 22.90 | 23.87 | 23.22 |
| Moisture, % | 8.95 | 8.89 | 8.68 |
| Crude fat, % | 7.22 | 7.20 | 7.64 |
| Crude fiber, % | 1.71 | 1.77 | 1.69 |
| Ash, % | 6.26 | 6.37 | 6.35 |
| Ca, % | 0.77 | 0.88 | 0.91 |
| P, % | 0.76 | 0.79 | 0.80 |

**Table 1.** Continued

[1]Diets were formulated to meet or exceed NRC (2012) recommendations. Phase 1 diets were fed from d 0 to d 14

[2] Control or peroxidized soybean oil. Peroxidation was created by heating control oil at 80ºC while bubbling air through oil at the rate of 50 L/min for 12 d. Control and peroxidized soybean oil were stabilized with 0.1% liquid antioxidant tertiary butyl hydroquinone (TBHQ) after peroxidation

[3]Supplied per kg of complete diet: 33 mg of Mn, 110 mg of Zn, 110 mg of Fe, 17 mg of Cu, 0.30 mg of I and 0.30 mg of Se

[4]Supplied per kg of complete diet: 9,700 IU of vitamin A, 871 IU of vitamin $D_3$, 66.1 IU of vitamin E, 0.03 mg of vitamin $B_{12}$ as d-calcium pantothenate, 3.6 mg of vitamin K as menadione sodium bisulfite complex, 11 mg of riboflavin, 27.6 mg of d-pantothenic acid, 44.1 mg of niacin, 2.5 mg of vitamin $B_6$ as pyridoxine hydrochloride, 2.6 mg of folic acid and 0.44 mg of biotin.

[5] Rovimix®, DSM, Heerlen, The Netherlands

[6] Analyzed by Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO

**Table 2.** Composition of the experimental Phase 2diets. As fed basis[1]

| | Treatments | | |
|---|---|---|---|
| Item | **CON** | **VITE** | **POL** |
| **Ingredient, %** | | | |
| Corn, yellow dent | 55.34 | 55.34 | 55.34 |
| Soybean meal, 47.5% CP | 34.67 | 34.67 | 34.67 |
| Soybean oil[2] | 6.00 | 6.00 | 6.00 |
| Monocalcium phosphate 21% | 1.53 | 1.53 | 1.53 |
| Limestone | 1.16 | 1.16 | 1.16 |
| Copper sulfate, 25.2% Cu | 0.07 | 0.07 | 0.07 |
| L-lysine HCl 78.5% | 0.36 | 0.36 | 0.36 |
| DL-methionine | 0.18 | 0.18 | 0.18 |
| L-threonine | 0.14 | 0.14 | 0.14 |
| Salt | 0.35 | 0.35 | 0.35 |
| Mineral premix[3] | 0.15 | 0.15 | 0.15 |
| Vitamin premix[4] | 0.05 | 0.05 | 0.05 |
| Vitamin E, IU/kg[5] | - | 200.00 | - |
| Polyphenols, mg/kg | - | - | 400.00 |
| **Calculated Composition** | | | |
| ME, kcal/kg | 3556 | 3556 | 3556 |
| Crude Protein % | 21.70 | 21.70 | 21.70 |
| SID Amino acids | | | |
| Lys % | 1.30 | 1.30 | 1.30 |
| Thr % | 0.81 | 0.81 | 0.81 |
| Met % | 0.47 | 0.47 | 0.47 |
| Met+Cys% | 0.75 | 0.75 | 0.75 |
| Trp % | 0.23 | 0.23 | 0.23 |
| Total Lysine % | 1.45 | 1.45 | 1.45 |
| **Analyzed composition[6]** | | | |
| Crude protein, % | 20.48 | 20.48 | 20.48 |
| Moisture, % | 11.68 | 11.68 | 11.68 |
| Crude fat, % | 4.67 | 4.67 | 4.67 |
| Crude fiber, % | 2.27 | 2.27 | 2.27 |
| Ash, % | 5.09 | 5.09 | 5.09 |
| Ca, % | 0.79 | 0.79 | 0.79 |
| P, % | 0.67 | 0.67 | 0.67 |

[1]Diets were formulated to exceed NRC (2012) requirements. Phase 2 diets were fed from d 15 to d 35

[2] Control or peroxidized soybean oil. Peroxidation was created by heating control oil at 80ºC while bubbling air through oil on the rate of 50L/min for 12 d. Control and peroxidized soybean oils were stabilized 0.1% liquid antioxidant tertiary butyl hydroquinone (TBHQ) after peroxidation

[3] Supplied per kg of complete diet: 33 mg of Mn, 110 mg of Zn, 110 mg of Fe, 17 mg of Cu, 0.30 mg of I and 0.30 mg of Se

[4]Supplied per kg of complete diet: 9700 IU of vitamin A, 871 IU of vitamin $D_3$, 66.1 IU of vitamin E, 0.03 mg of vitamin $B_{12}$ as d-calcium pantothenate, 3.6 mg of vitamin K as menadione sodium bisulfite complex, 11 mg of riboflavin, 27.6 mg of d-pantothenic acid, 44.1 mg of niacin, 2.5 mg of vitamin $B_6$ as pyridoxine hydrochloride, 2.6 mg of folic acid and 0.44 mg of biotin.

**Table 2.** Continued

[5] Rovimix®, DSM, Heerlen, The Netherlands
[6] Analyzed by Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO

**Table 3.** Analyzed composition of experimental soybean oil sources[1]

| Item | **Control** | **Peroxidized** |
|---|---|---|
| Moisture, % | 0.10 | 0.11 |
| Insoluble impurities, % | 0.06 | none |
| Unsaponifiable matter, % | 0.38 | 0.34 |
| Free fatty acids, % | 0.02 | 0.09 |
| Peroxide Value, mEq/kg fat | | |
| Initial[2] | 4.1 | 141.6 |
| 4 hr AOM[3] | 3.6 | 158 |
| 20 hr AOM | 4.6 | 41.2 |
| Anisidine value | 1.7 | 106 |
| Oxidative Stability Index (OSI), hours[4] | 37 | 2.4 |
| Hexanal, ppm[4] | <5 | 99 |
| 2, 4-Decadienal, ppm[4] | 8 | 720 |
| Antioxidant, % | 0.1 | 0.1 |

[1]Analyzed by New Jersey Feed Laboratory Inc., Trenton, NJ
[2]Analyzed by Kemin Industries, Inc. Des Moines, IA and New Jersey Feed Laboratory Inc., Trenton, NJ, both analyzed initial peroxide value and the mean is reported here.
[3]AOM: Active oxygen method
[4]Analyzed by Kemin Industries, Inc. Des Moines, IA

**Table 4.** Growth performance of piglets fed dietary control or peroxidized oil with addition or not of antioxidants over a 35-day period[1]

| | Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control oil | | | Peroxidized oil | | | | *P*-value[2] | | |
| Item | CON | VITE | POL | CON | VITE | POL | SEM | O | S | O x S |
| BW, kg | | | | | | | | | | |
| d0 | 6.62 | 6.63 | 6.61 | 6.61 | 6.62 | 6.62 | 0.390 | 0.880 | 0.600 | 0.792 |
| d7 | 7.63 | 7.44 | 7.40 | 7.52 | 7.38 | 7.57 | 0.390 | 0.982 | 0.128 | 0.189 |
| d14 | 10.23 | 10.06 | 10.24 | 9.81 | 9.82 | 10.25 | 0.410 | 0.129 | 0.193 | 0.445 |
| d21 | 13.4 | 13.0 | 13.2 | 12.0 | 11.8 | 12.7 | 0.478 | <0.001 | 0.110 | 0.254 |
| d28 | 17.6 | 17.3 | 17.5 | 14.8 | 14.6 | 15.8 | 0.613 | <0.001 | 0.272 | 0.414 |
| d35 | 23.3 | 23.0 | 23.2 | 18.4 | 18.3 | 19.5 | 0.736 | <0.001 | 0.356 | 0.489 |
| ADG, kg | | | | | | | | | | |
| d 0 to 7 | 0.145 | 0.117 | 0.112 | 0.130 | 0.108 | 0.136 | 0.013 | 0.975 | 0.107 | 0.218 |
| d 7 to 14 | 0.372 | 0.374 | 0.396 | 0.327 | 0.344 | 0.383 | 0.020 | 0.045 | 0.063 | 0.654 |
| d 14 to 21 | 0.450 | 0.413 | 0.423 | 0.311 | 0.279 | 0.347 | 0.031 | <0.001 | 0.219 | 0.364 |
| d 21 to 28 | 0.602 | 0.615 | 0.613 | 0.400 | 0.399 | 0.443 | 0.036 | <0.001 | 0.732 | 0.806 |
| d 28 to 35 | 0.814 | 0.814 | 0.821 | 0.509 | 0.538 | 0.537 | 0.031 | <0.001 | 0.788 | 0.851 |
| ADG, kg (phases) | | | | | | | | | | |
| Phase 1 | 0.258 | 0.245 | 0.258 | 0.229 | 0.228 | 0.260 | 0.015 | 0.133 | 0.180 | 0.442 |
| Phase 2 | 0.622 | 0.614 | 0.619 | 0.407 | 0.405 | 0.442 | 0.023 | <0.001 | 0.586 | 0.625 |
| Overall ADG | 0.477 | 0.467 | 0.475 | 0.336 | 0.334 | 0.369 | 0.018 | <0.001 | 0.357 | 0.492 |
| ADFI, kg | | | | | | | | | | |
| d 0 to 7 | 0.194 | 0.167 | 0.167 | 0.182 | 0.167 | 0.177 | 0.012 | 0.930 | 0.171 | 0.626 |
| d 7 to 14 | 0.447 | 0.437 | 0.461 | 0.413 | 0.416 | 0.440 | 0.019 | 0.093 | 0.347 | 0.914 |
| d 14 to 21 | 0.786 | 0.785 | 0.764 | 0.657 | 0.695 | 0.676 | 0.036 | <0.001 | 0.810 | 0.798 |
| d 21 to 28 | 0.851 | 0.861 | 0.832 | 0.619 | 0.654 | 0.692 | 0.037 | <0.001 | 0.746 | 0.454 |
| d 28 to 35 | 1.194 | 1.165 | 1.135 | 0.884 | 0.903 | 0.901 | 0.039 | <0.001 | 0.804 | 0.526 |
| ADFI, kg (phases) | | | | | | | | | | |
| Phase 1 | 0.321 | 0.302 | 0.317 | 0.297 | 0.294 | 0.308 | 0.014 | 0.219 | 0.511 | 0.798 |

**Table 4.** Continued

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Phase 2 | 0.906 | 0.888 | 0.872 | 0.671 | 0.695 | 0.709 | 0.031 | <0.001 | 0.995 | 0.426 |
| Overall ADFI | 0.672 | 0.653 | 0.650 | 0.521 | 0.535 | 0.549 | 0.022 | <0.001 | 0.966 | 0.450 |
| G: F | | | | | | | | | | |
| d 0 to 7 | 0.746 | 0.692 | 0.666 | 0.709 | 0.633 | 0.759 | 0.042 | 0.963 | 0.181 | 0.084 |
| d 7 to 14 | 0.833 | 0.852 | 0.852 | 0.788 | 0.829 | 0.872 | 0.028 | 0.379 | 0.112 | 0.547 |
| d 14 to 21 | 0.568 | 0.523 | 0.553 | 0.469 | 0.406 | 0.513 | 0.030 | <0.001 | 0.019 | 0.250 |
| d 21 to 28 | 0.710 | 0.713 | 0.735 | 0.642 | 0.605 | 0.643 | 0.030 | 0.001 | 0.599 | 0.798 |
| d 28 to 35 | 0.686 | 0.699 | 0.726 | 0.577 | 0.597 | 0.601 | 0.029 | <0.001 | 0.520 | 0.915 |
| G: F | | | | | | | | | | |
| Phase 1 | 0.806 | 0.809 | 0.812 | 0.764 | 0.777 | 0.842 | 0.027 | 0.427 | 0.151 | 0.241 |
| G: F | | | | | | | | | | |
| Phase 2 | 0.688 | 0.693 | 0.712 | 0.605 | 0.580 | 0.627 | 0.021 | <0.001 | 0.241 | 0.705 |
| Overall | | | | | | | | | | |
| G:F | 0.710 | 0.714 | 0.732 | 0.641 | 0.624 | 0.675 | 0.018 | <0.001 | 0.074 | 0.556 |

[1]Values represent least squares means of 8 pens with 4 pigs (2 gilts and 2 barrows) per pen
[2]Effects abbreviations: O = oil, S = supplementation, O x S = oil x supplementation

**Table 5.** Serum vitamin E of piglets fed dietary control or peroxidized oil with addition or not of antioxidants measured on d 14 and d 35[1]

| | | Treatments | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control oil | | | Peroxidized oil | | | | *P* – value | | | | | |
| **Item** | **Day** | **CON** | **VITE** | **POL** | **CON** | **VITE** | **POL** | **SEM** | **O** | **S** | **D** | **O x S** | **O x D** | **S x D** |
| Vitamin E | | | | | | | | | | | | | | |
| Serum, ppm | 14 | 1.61 | 2.59 | 1.65 | 1.35 | 1.54 | 1.26 | 0.087 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.082 |
| | 35 | 1.31 | 2.80 | 1.35 | 0.71 | 0.98 | 0.75 | | | | | | | |

[1]Values represent least squares means of 8 pigs (either gilt or barrow)
[2]Effects abbreviations: O = oils, S = supplementation, O x S = oil x supplementation, D = Day, O x D = Oil x day, S x D = supplementation x day. Three-factor interaction was not significant.

**Table 6.** Oxidative stress and antioxidant capacity status in serum of pigs fed control or peroxidized oil with addition or not of antioxidants measured on d 14 and d 35[1]

| | | Treatments | | | | | | | *P*-value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control oil | | | Peroxidized oil | | | | | | | | | | |
| **Item** | **Day** | **CON** | **VITE** | **POL** | **CON** | **VITE** | **POL** | **SEM** | **O** | **D** | **S** | **O x D** | **O x S** | **S x D** | **O x S x D** |
| 8-OHdG[3], ng/mL | 14 | 5.1 | 5.5 | 5.1 | 4.6 | 5.1 | 4.2 | 0.786 | 0.128 | 0.653 | 0.705 | 0.458 | 0.298 | 0.513 | 0.856 |
| | 35 | 4.8 | 4.9 | 5.7 | 4.8 | 5.3 | 4.8 | | | | | | | | |
| Protein Carbonyl, pmol/mg protein | 14 | 2.09 | 1.91 | 1.93 | 1.89 | 2.24 | 2.10 | 0.215 | <0.001 | <0.001 | 0.446 | <0.001 | 0.231 | 0.553 | 0.099 |
| | 35 | 2.28 | 2.58 | 2.37 | 3.25 | 3.11 | 3.69 | | | | | | | | |
| MDA[4], µM | 14 | 11.7 | 13.9 | 12.5 | 8.6 | 8.3 | 8.4 | 0.887 | <0.001 | 0.004 | 0.165 | 0.001 | 0.132 | 0.457 | 0.460 |
| | 35 | 11.2 | 12.2 | 14.1 | 11.9 | 12.0 | 12.1 | | | | | | | | |
| TAC[5], CRE[6]/mL | 14 | 288 | 323 | 303 | 295 | 255 | 261 | 15.867 | <0.001 | <0.001 | 0.538 | 0.924 | 0.045 | 0.025 | 0.305 |
| | 35 | 335 | 355 | 379 | 317 | 310 | 335 | | | | | | | | |

[1]Values represent least squares means of 8 pigs (either gilt or barrow)
[2]Effects abbreviations: O = oils, D=day, O x D= oil x day, S = supplementation
[3]8-OHdG: 8-hydroxydeoxyguanosine
[4]MDA: malondialdehyde
[5]TAC: total antioxidant capacity
[6]CRE: copper reducing equivalent

**Table 7.** Immune stress status in serum of pigs fed control or peroxidized oil with addition or not of antioxidants measured on d 14 and d 35[1]

| | | Treatments | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control oil | | | Peroxidized oil | | | | *P* – value[2] | | | | |
| **Item** | **Day** | **CON** | **VITE** | **POL** | **CON** | **VITE** | **POL** | **SEM** | **O** | **D** | **S** | **O x D** | **O x S x D** |
| IFN-γ[3], pg/mL | | | | | | | | | | | | | |
| | 14 | 14510 | 4936 | 15596 | 10765 | 6032 | 7193 | 3330.16 | 0.408 | 0.034 | 0.116 | 0.158 | 0.337 |
| | 35 | 14757 | 7987 | 14824 | 11460 | 10368 | 14351 | | | | | | |
| IL-1α[4], pg/mL | | | | | | | | | | | | | |
| | 14 | 132 | 96 | 97 | 73 | 56 | 63 | 32.17 | 0.601 | 0.017 | 0.351 | 0.009 | 0.703 |
| | 35 | 47 | 36 | 32 | 84 | 77 | 4 | | | | | | |
| IL-1β[5], pg/mL | | | | | | | | | | | | | |
| | 14 | 2030 | 1139 | 891 | 612 | 415 | 518 | 350.41 | 0.307 | 0.052 | 0.043 | 0.003 | 0.230 |
| | 35 | 388 | 337 | 247 | 735 | 1092 | 394 | | | | | | |
| IL-1ra[6], pg/mL | | | | | | | | | | | | | |
| | 14 | 1254 | 861 | 737 | 637 | 615 | 674 | 159.5 | 0.939 | 0.022 | 0.083 | 0.002 | 0.239 |
| | 35 | 507 | 414 | 369 | 932 | 635 | 607 | | | | | | |
| IL-2[7], pg/mL | | | | | | | | | | | | | |
| | 14 | 1412 | 406 | 739 | 529 | 377 | 489 | 202.66 | 0.481 | 0.01 | 0.052 | 0.015 | 0.149 |
| | 35 | 244 | 235 | 203 | 642 | 438 | 259 | | | | | | |
| IL-4[8], pg/mL | | | | | | | | | | | | | |
| | 14 | 8055 | 2040 | 5686 | 3931 | 2307 | 2403 | 1342.54 | 0.774 | 0.018 | 0.056 | 0.010 | 0.357 |
| | 35 | 1391 | 1162 | 901 | 4510 | 2956 | 1747 | | | | | | |
| IL-6[9], pg/mL | | | | | | | | | | | | | |
| | 14 | 254 | 154 | 155 | 100 | 73 | 85 | 45.57 | 0.361 | 0.006 | 0.420 | 0.005 | 0.590 |
| | 35 | 43 | 34 | 30 | 85 | 140 | 37 | | | | | | |
| IL-8[10], pg/mL | | | | | | | | | | | | | |
| | 14 | 623 | 757 | 985 | 427 | 507 | 673 | 179.69 | 0.135 | 0.827 | 0.801 | 0.422 | 0.799 |
| | 35 | 672 | 821 | 730 | 755 | 607 | 491 | | | | | | |

**Table 7.** Continued

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IL-10[11], pg/mL | | | | | | | | | | | | |
| | 14 | 3840 | 2519 | 2450 | 1634 | 1124 | 1451 | 656.67 | 0.142 | 0.001 | 0.317 | 0.02 | 0.544 |
| | 35 | 782 | 566 | 623 | 1464 | 1063 | 495 | | | | | | |
| IL-12[12], pg/mL | | | | | | | | | | | | |
| | 14 | 1588 | 1310 | 1193 | 996 | 1044 | 998 | 147.45 | 0.198 | 0.004 | 0.263 | 0.008 | 0.092 |
| | 35 | 803 | 817 | 958 | 1232 | 877 | 873 | | | | | | |
| IL-18[13], pg/mL | | | | | | | | | | | | |
| | 14 | 3384 | 1617 | 2450 | 1871 | 1352 | 1642 | 542.48 | 0.764 | 0.021 | 0.118 | 0.021 | 0.459 |
| | 35 | 1104 | 963 | 787 | 2265 | 1585 | 1005 | | | | | | |
| TNF-α[14], pg/mL | | | | | | | | | | | | |
| | 14 | 23 | 251 | 91 | 199 | 35 | 16 | 59.44 | 0.428 | 0.419 | 0.885 | 0.794 | 0.023 |
| | 35 | 89 | 42 | 131 | 59 | 69 | 70 | | | | | | |

[1]Values analyzed represent least squares means of 8 pigs (either gilt or barrow)
[2]Effects abbreviations: O = oils, D=day, O x D= oil x day, S = supplementation; O x S = oil x supplementation ($P \geq 0.05$) and S x D = supplementation x day ($P \geq 0.05$)
[3] IFN-γ: interferon-gamma
[4] IL-α: interleukin-alpha
[5] IL-β: interleukin-beta
[6] IL-1ra: interleukin-1receptor antagonist
[7] IL-2: interleukin-2
[8]IL-4: interleukin-4
[9]IL-6: interleukin-6
[10]IL-8: interleukin-8
[11]IL-10: interleukin-10
[12]IL-12: interleukin-12
[13]IL-18: interleukin-18
[14]TNF-α: tumor necrosis factor - alpha

# CHAPTER IV:

## Effects of addition of vitamin E and phytogenic compounds in drinking water on growth performance, oxidative stress and immune status of newly weaned pigs

**ABSTRACT:** The objective of the present study was to evaluate the use of vitamin E and phytogenic compounds in drinking water on performance, oxidative stress, and immune status of weaned piglets fed peroxidized lipids. In a 35-day study, 21-d old weaned piglets (*n*=96; 6.10 ± 0.64 kg BW) were assigned within sex and BW blocks to 1 of 4 dietary treatments, using 24 pens (4 pigs/pen; 6 replications/treatment). Diets contained either 6% human-grade soybean oil or 6% soybean oil which was peroxidized for 12 d at 80°C with constant air flow at 50 L/min (initial peroxide value, anisidine value, hexanal, and 2,4-decadienal concentrations were 4.1 vs. 141.6 meq/kg, 1.7 vs. 106, <5 vs. 99 mg/kg, and 8 vs. 720 mg/kg for control and peroxidized oil, respectively). Pigs fed peroxidized lipids received drinking water without (control) or with supplemental vitamin E (100 IU/L of d-α-tocopherol) or phytogenics (60 µL/L from d 1-7 and 30 µL/L from d 8-35). Pigs fed control diet received control water only. Overall, peroxidized lipids decreased ($P < 0.001$) BW (18.20 vs. 21.55 kg) and ADG (347 vs. 441 g/day) and tended to decrease ADFI ($P = 0.14$; 537 vs. 617 g/day) and G: F ($P = 0.07$; 645 vs. 715 g/kg). Peroxidation decreased serum vitamin E ($P = 0.03$) which could be restored ($P=0.01$) by vitamin E in the water, but not phytogenics. Serum concentrations of interferon-γ, interleukin (IL)-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, TNF-α, malondialdehyde, protein carbonyl, and total antioxidant capacity were not impacted by treatments ($P > 0.05$). Serum 8-hydroxydeoxyguanosine was reduced ($P = 0.001$) with feeding peroxidized lipids and this was not altered by supplemental antioxidants ($P > 0.45$). Peroxidized lipids clearly reduced growth performance, which did not appear to be related to oxidative stress markers or immune-regulatory cytokines. The negative effects of peroxidized lipids could not be improved by vitamin E or phytogenics supplemented in drinking water.

**Key words:** antioxidants, vitamin E, phytogenic compounds, weaned pigs

## Introduction

Deep-frying is a common cooking method used around the world. It uses vegetable oils, such as palm, soybean oil, canola, or sunflower oil under high temperature (Santos *et al*., 2018) to cook a variety of food items. After cooking, used vegetable oils may be discarded (Singh-Ackbarali *et al*., 2017), or they can be used for livestock diets (Blas *et al*., 2010; Panadare and Rathod, 2015; Shurson *et al*., 2015; and Peixoto *et al*., 2017). Used oils treated by heat produce peroxidized lipids which is caused by attack of oxygen molecules on polyunsaturated fatty acids (Kerr *et al*., 2015).

Peroxidized oils can decrease body weight gain in piglets (DeRouchey *et al*., 2004; Liu *et al*., 2014; Rosero *et al*., 2015), feed intake in pigs and poultry (Hung *et al*., 2017), gain:feed efficiency (DeRouchey *et al*., 2004; Lu *et al*., 2014; Rosero *et al*., 2015), and can increase mortality and the number of viable pigs (Chang *et al*., 2019). Also, damage to intestinal morphology was reported, leading to decreased intestinal absorptive capacity and a disruption of the intestinal redox system (Rosero *et al*., 2015). The alteration of the balance between antioxidants and oxidants can cause cellular oxidative stress and activation of the immune system (Lu *et al*., 2014).

Natural antioxidants in the diets for pigs are considered viable options to reduce oxidative stress, recovering the antioxidant balance in pigs (Sies, 1992). Vitamin E plays an antioxidant role in the body and is considered one of the most powerful antioxidants (Sies, 1992). Vitamin E protects lipids from free radical attack, scavenging lipid peroxyl radicals (Lúcio *et al*., 2009) and was used to prevent oxidative stress in livestock (Brenes *et al*., 2008; and Gerasopoulos *et al*., 2015).

Phytogenics can have antioxidant properties and are currently used in diets for pigs. They include essential oils, spices and herbal extracts (Windish *et al*., 2008). Phytogenics can reduce

oxidative stress due to functional antioxidant capacity in pigs (Zhang *et al*., 2015) and can improve palatability of feed (Yang *et al*., 2015).

Weaning negatively impacts piglets and feed consumption may be completely lacking for several days after weaning. However, drinking water consumption is increased in compensation of the reduction in feed intake during first days post-weaning (Moeser *et al*., 2017). To take advantage of this higher predisposition by piglets to consume water during the first days postweaning, the objective of the present experiment was to evaluate the supplementation of vitamin E or phytogenics in the drinking water for newly weaned piglets on growth performance, oxidative and immune status when using dietary peroxidized oils.

**Materials and methods**

The experimental protocol and procedures were reviewed and approved by the Institutional Animal Care and Use Committee of North Carolina State University (Raleigh, NC, US).

***Animals and experimental design***

A study was conducted at the Swine Educational Unit (Raleigh, NC). For the study, 96 crossbred pigs were used. Pigs were 3 weeks old, half barrows and gilts, with an initial body weight of 6.10 ± 0.64 kg. Piglets, at weaning, were blocked by initial body weight and sex and randomly assigned within blocks to 1 of 4 dietary treatments using an experimental allotment program (Kim and Lindemann, 2007). Four dietary treatments were used as follows: T1: control diet + 6% soybean oil; T2: control diet + 6% peroxidized soybean oil; T3: T2 + 100 IU/L of d-α-tocopherol (Emcelle® tocopherol, Stuart products, Bedford Road, Bedford, TX); and T4: T2 + 60 µL/L from d 0 to 7 and 30 µL/L from d 8 to 35 of a commercial phytogenic product. The phytogenic product was an oily product containing oregano, essential oil of citrus peels, essential oil of anise, and mono-and diglycerides of edible fat forming acids.

The stock to prepare the vitamin E treatment was made by dilution of the vitamin E product in drinking water at the ratio of 0.0256:1. The vitamin E stock solution was subsequently metered into the drinking water system at a rate of 1:128 vitamin E stock solution:drinking water using a water medication device (Dosatron DM11F, Hog Slat, Newton Grove, NC). The vitamin E stock was provided to respective pens through the water system previously installed at the farm. Treated water was supplied to randomly selected pens (6 pens per treatment).

To prepare the stock for the phytogenic treatment, 7.68 mL product per 1 L of drinking water for d 1 to 7, and 3.84 mL of product per 1 L of drinking water for d 8 to 35 was used to create the stock solution. The phytogenic stock was metered into the drinking water system at a rate of 1:128 phytogenic stock solution:drinking water using a water medication device (Dosatron DM11F, Hog Slat, Newton Grove, NC).The phytogenic stock was provided to randomly selected pens via the water system previously installed at the farm. The final dilution provided to the pigs for d 1 to 7 was 60 µL per L of drinking water, and 30 µL per L for d 8 to 35.

To create the peroxidized oil, 163 kg of human-grade soybean oil (Kirkland, Washington, WA) was equally divided into two metal barrels. The barrels each contained a heater and a thin PVC tube with 1 mm holes to pump air inside of each barrel. The heater was active for 12 days at 80°C and air was provided continuously at a constant air flow of 50 L/min. The control and peroxidized oil were used to make diets two days after the peroxidation was completed.

The dietary treatment feeds were manufactured at the North Carolina State University Feed Mill Educational Unit (Raleigh, NC). Diets were based on corn-soybean meal for phase 1 and phase 2 and they were formulated to meet or exceed all nutrient requirements for piglets (Table 1 and 2, respectively) as suggested by the NRC (2012). At the same day of batching, 0.1% of liquid antioxidant (Rendox®, Kemin industries, Inc. Des Moines, IA) containing tertiary butyl

hydroquinone (TBHQ) was added to the oil sources to prevent further oxidation of the lipids. Batches for phase 1 and phase 2 were made on different days to provide fresh feed to the pigs. Within each phase, a basal mix was created first, containing all ingredients with the exception of the lipid sources and the test ingredients. The basal mix was divided into 2 batches. The first batch was mixed with 6% of normal soybean oil and the second batch included 6% peroxidized soybean oil. Pigs were fed diets in 2 phases, with phase fed from d 0 for 14 days and phase 2 for the next 21 days. All piglets were provided *ad libitum* access to feed and drinking water.

Pigs were allotted into a temperature-controlled room (Aerotech® AeroSpeed 1.2, Pittsburgh, PA) to 24 pens (pen: size 0.91 m x 1.52 m) and housed four pigs per pen (2 gilts and 2 barrows). Each pen contained a stainless-steel pig feeder, single-sided with 2 feeding spaces (Staco® Inc, Schaeffer town, PA) and two stainless water nipples per pen. Environmental temperatures and ventilation were checked every morning and set at 32 °C for the first week and reduced by 2°C every week until the set point reached 24°C.

***Growth performance***

Body weight (BW) was measured on d 0, 7, 14, 21, 28 and 35 to calculate average daily gain (ADG). Daily feed intake was measured from the difference between daily feed additions and feed remaining at the end of the week and divided by 7 days and for each phase. Feed efficiency (G: F) was calculated by dividing ADG by ADFI weekly and for each phase.

***Serum sample collection***

Blood samples from one pig per pen were collected by venipuncture (jugular vein) using 20-gauge x 3.8 cm multiple use drawing needles (Vacuette, Greiner bio-one, Kremsmunster, Austria) on d 7 and d 35 (at 0900). Blood for serum analysis was collected into two 10 mL-vacuum tubes (BD Vacutainer Serum, Franklin Lakes, NJ). Blood was centrifuged using a refrigerated

centrifuge (Centra GP8R, Thermo IEC, MA) to collect serum supernatant at 4,267 x g for 10 min at 4°C. The supernatant was aliquoted into 6 tubes of 2 mL capacity (Biotix®, Neptune, 3472.X, Mesa Rim, San Diego, CA) and stored at -80°C for further analysis.

***Chemical Analysis***

Proximate analysis of the diets was determined by Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO) using AOAC Official method (2006). Diets were analyzed for moisture (Method 934. 01), crude protein (Method 990. 03), crude fat (Method 920. 39 (A)), crude fiber (Method 978. 10), ash (Method 942.05), neutral detergent fiber (JAOAC 56, 1352-1356, 1973) acid detergent fiber (Method 973. 18 (A-D)), calcium (Method 985.01 (A, B, D)) and phosphorus (Method 966. 01).

Oil samples from control and peroxidized oil were collected and analyzed by New Jersey Feed Laboratory Inc., (Trenton, NJ) using AOAC. (1990) and AOCS (1998) procedures, and by Kemin Industries, Inc. (Des Moines, IA) (Table 3).

***Vitamin E concentration in serum***

Frozen serum samples collected at d 7 and 35 were submitted to the Veterinary Diagnostic Laboratory at Iowa State University (Ames, IA) for the analysis of vitamin E. Samples were analyzed using high performance liquid chromatography methods.

***Immune status in serum***

Frozen serum samples from d 7 and d 35 were submitted to Eve Technologies Corporation (Calgary, Canada) for analysis of pro- and anti-inflammatory cytokines using Luminex xMAP Multi-plex technique (multiplex technology). Results of cytokines from IFN (interferon)-$\gamma$, IL (interleukin)-1$\alpha$, IL-1$\beta$, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and TNF (tumor necrosis factor)-$\alpha$ were reported and expressed in pg/mL of serum.

***Oxidative status in serum***

Malondialdehyde (MDA) was measured in the serum samples for d 7 and 35 using Oxiselect™ TBARS Assay Kit (MDA Quantitation; Cell BioLabs, Inc., San Diego, CA) catalog number STA330. Absorbance was measured at 532 nm on a microplate reader (Bio Tek Instruments®, Synergy HT, Winooski, VT) and using a software program KC4™, Bio Tek Instruments®, Winooski, VT). Results from MDA for serum samples were expressed in µM. The intra assay CV was 5%.

8-hydroxydeoxyguanosine (8-OHdG) was measured in serum samples collected on d 7 and 35 using Oxiselect™ Oxidative DNA Damage ELISA kit (8-OHdG Quantitation; Cell BioLabs, Inc., San Diego, CA) catalog number STA320. Absorbance was measured at 450 nm on a microplate reader (Bio Tek Instruments®, Synergy HT, Winooski, VT) and using a software program (KC4™ Bio Tek Instruments®, Winooski, VT). Results were expressed in ng/mL. Intra- and inter-essay CV were 3 and 1%, respectively.

Protein carbonyl was measured in serum samples collected on d 7 and 35 using protein carbonyl colorimetric assay kit (Cayman Chemical®, Ann Arbor, MI). Protein content on the samples was measured using a BCA protein assay kit (Fisher Scientific®, Hampton, NH). Absorbance was measured at 360 nm on a microplate reader (Bio Tek Instruments®, Synergy HT, Winooski, VT) and using a software program (KC4™, Bio Tek Instruments®, Winooski, VT). Results were expressed in pmol carbonyl/mg of protein. Intra-and inter-essay CV were 7 and 9 %, respectively.

***Antioxidant activity***

Total antioxidant capacity (TAC) was measured for serum samples collected on d 7 and 35 using the Oxiselect™ Total Antioxidant Capacity (TAC) assay kit (Cell BioLabs, Inc., San Diego,

CA) catalog number STA360. Absorbance was measured at 490 nm on a microplate reader (Bio Tek Instruments®, Synergy HT, Winooski, VT) and using a software program (KC4™, Bio Tek Instruments®, Winooski, VT). Results were expressed as µM Copper Reducing Equivalents (CRE). CRE sample values are proportional to the sample's total antioxidant capacity. Intra-and inter-assay CV were 3% and 2.7%, respectively.

***Statistical analysis***

Data for growth performance were analyzed using the GLIMMIX Procedure of SAS v.9.4 (SAS Institute. Inc., Cary, NC). Pen was used as the experimental unit and weight block was the random effect. The statistical model included block, type of oil and water supplementation treatment. For serum measurements, pig was considered the experimental unit and the MIXED Procedure of SAS was used. The statistical model included block, type of oil, water supplementation treatments, day of collection, and their interactions. Statistical significances were considered at $P < 0.05$ and tendencies at $0.05 \leq P \leq 0.10$.

**Results**

***Chemical analysis of peroxidized soybean oil***

Results of chemical analysis of peroxidized and control soybean oil are shown in Table 3. Initial peroxide value, 4 h AOM, 20 h AOM, anisidine value, hexanal and 2, 4-decadienal were increased in peroxidized oil (4.1 vs. 141.6 mEq/kg fat; 3.6 vs. 158;4.6 vs. 41.2; 1.7 vs. 106; <5 vs. 99 ppm and 8 vs. 720 ppm). Oxidative stability index was decreased on peroxidized oil (37 vs. 2.4 h).

***Growth performance***

Three pigs, one from a pen of dietary peroxidized oil supplemented with phytogenics in water, one from a pen of dietary control oil treatment, and one from another pen of dietary control

oil treatment were removed from the study due to losses in BW on d 12, 15, and 16, respectively. One pig from the dietary peroxidized oil supplemented with phytogenics in water treatment died on d 33.

Peroxidized oil treatments reduced ($P < 0.05$) BW on d 28 (-11 %) and d 35 ($P < 0.001$; -15.4 %) compared to control oil (Table 4). ADG during week 3 ($P < 0.05$; -21 %), 4, 5, phase 2, and overall ($P < 0.001$; -32, -31, -28, and -22%, respectively) was reduced when peroxidized oil was fed compared with control oil). ADFI was reduced ($P < 0.05$) for peroxidized oil treatments on week 3, and 4 (-14, -18%, respectively), and tended to be reduced by peroxidized oil during phase 2 ($P = 0.061$). G:F was reduced ($P < 0.05$) for peroxidized oil with vitamin E when compared to control oil treatment for week 4 (-22.5%) and overall (-13%). Peroxidized oil and peroxidized oil with vitamin E treatments significatively reduced ($P < 0.05$) G:F when compared with control oil treatment during week 5 and phase 2.

***Vitamin E in serum***

Vitamin E concentration in serum was decreased ($P = 0.012$; -39 %) when measured on d 35 compared to d 7 (Table 5). Feeding of peroxidized lipids decreased serum concentrations of vitamin E compared to control oil and vitamin E supplementation in water of pigs fed peroxidized oil increased vitamin E in serum ($P < 0.05$). Supplementation of phytogenics did not impact serum vitamin E (Figure 1).

***Oxidative status in serum***

Both phytogenic supplementation in water and peroxidized oil without supplementation treatments decreased ($P < 0.05$) 8-OHdG when compared with control oil treatment (Table 6 and Figure 2). On d 35, serum protein carbonyl was increased ($P = 0.011$) by 24% compared to d 7 and serum protein carbonyl was increased on d 35 for the peroxidized control oil treatment and the

peroxidized oil with phytogenic supplementation, but not for control oil treatment or peroxidized oil with vitamin E treatment ($P$ = 0.029). The concentration of MDA in serum increased ($P$ = 0.003; +29 %) on d 35 compared with d 7.

***Antioxidant activity***

Serum TAC increased ($P$ < 0.001; +25 %) on d 35 when compared to d 7 (Table 6). Furthermore, TAC in serum was decreased ($P$ = 0.099) by peroxidized oil treatments in comparison with the normal oil treatment.

***Immune status in serum***

Pro- and anti-inflammatories cytokines (IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18) were not impacted ($P \geq 0.05$) by day of collection or treatments. (Table 7). IL-1ra and IL-8 were reduced (-43 and -43%, respectively) on d 35 when compared with d 7, but they were not different between treatments. The peroxidized oil diet with phytogenics tended ($P$ = 0.068) to increase TNF-α in comparison with the other treatments, but no other differences were detected.

**Discussion**

Peroxidation of oils is produced by high temperatures and oxygen molecules in the air, forming radicals. Light, metals and reactive oxygen species (ROS) facilitate the formation of rancid oil (Choe and Min, 2007). Thermally peroxidized oil can negatively impact growth performance in pigs, as observed in the present study. Growth performance was affected principally in the last three weeks due to feeding dietary peroxidized oil. Feed intake started to be affected during the third week, causing significantly decreased BW and ADG and this continued during the fourth, and fifth week, and overall. The addition of vitamin E or phytogenics in the drinking water did not ameliorate the negative impact of dietary peroxidized oil. Similar to our study, Boler *et al*. (2012), Rosero *et al*. (2015), Hanson *et al*. (2016) and Chang *et al*. (2019)

showed decreased growth performance when dietary peroxidized oil was included in diets for pigs. In the present study, G:F was negatively affected by the supplementation of vitamin E in the drinking water when dietary peroxidized oil was fed in comparison with the control oil treatment. In a study conducted by van Heugten *et al*. (1997), using vitamin E (24.2 µg/mL) and selenium (11 µg/mL) in the drinking water for 2 or 5 weeks after weaning, no differences were observed in ADG or ADFI, but was improved feed efficiency was reported when supplementation was provided for 5 weeks.. In that study, selenium was supplemented in the drinking water with vitamin E and it is well known that selenium is part of glutathione peroxidase, which catalyze the reduction of hydrogen peroxide to water (Liu *et al*., 2016). Further, vitamin E reduces free radicals, reinforcing the antioxidant system. Thus, both are components of the cellular antioxidant cycle having a direct impact on growth performance (Rooke *et al*., 2004) in pigs. The results in the present study did not demonstrate improvements in growth performance with supplementation of vitamin E alone. Perhaps supplementation of a variety of antioxidants is required to alleviate the negative impact of peroxidized lipids. Phytogenic compounds have antioxidant properties and they contribute to protect cells from lipid peroxidation caused by oxidation (Windisch *et al*., 2008). In the present study, the supplementation of phytogenics in the water of pigs fed peroxidized oil did not affect growth performance. Likewise, Schöne *et al*. (2006) and Botsoglou *et al*. (2004) reported no improvements in growth performance when dietary essential oils were used in piglets and broilers, respectively.

The role of vitamin E as antioxidant is inhibiting the oxidation process and producing cellular oxidation stability (Boler *et al*., 2012). In the present study, serum vitamin E was reduced on d 35 in comparison with d 7, suggesting that the natural antioxidant system and the vitamin E provided by the diet were used against oxidation due to postweaning stressors factors in all pigs

during that period. Likewise, the peroxidized oil supplemented with vitamin E in the water had high concentrations of serum vitamin E in comparison with peroxidized oil diet. Is clearly noted that the supplementation of vitamin E in water increased serum vitamin E. At the same time, the addition of phytogenics did not impact serum concentrations of vitamin E, suggesting that the addition of phytogenics in water did not help in regenerating vitamin E when peroxidized diets were fed. Moreover, van Heugten *et al*. (1997) reported increased serum vitamin E on d 7 when vitamin E and selenium were supplemented in drinking water for piglets. In contrast, Luehring *et al*. (2011) reported a reduction in plasma vitamin E when adding quercetin (phytogenics) to peroxidized fish oil diets with low intake of vitamin E.

Oxidative stress can be induced by dietary peroxidized oil, forming oxidative products that can damage DNA causing negative impacts on the transcription and replication of genes. A common oxidative marker is 8-hydroxydeoxyguanosine (8-OHdG) produced due to oxidation of guanosine by hydroxyl radical (Mateos and Bravo, 2007; and Pisoschi and Pop, 2015). DNA damage is caused by dietary peroxidized oil as reported by Lindblom *et al*. (2018) in tissue liver. However, in the present study serum 8-OHdG was increased in the control oil treatment when compared with the peroxidized oil treatment with or without phytogenics treatments. These results also contradict those reported by Chang *et al*. (2019), who did not observe differences in 8-OHdG using different peroxidized oil levels in piglets. The results of the present study suggested that the natural antioxidant system of pigs fed peroxidized oil reacted efficiently to cellular peroxidation, protecting DNA from oxidative damage.

Amino acids are sensitive to oxidative stress caused by peroxidation. When amino acids such as lysine, proline, arginine and threonine are oxidized, they form carbonyls, which are used as a marker of protein oxidation (Pisoschi and Pop, 2015). In the present study, protein carbonyl

increased on d 35 compared to d 7 and this increase in protein carbonyl can be related to the decrease in vitamin E on d 35. Additionally, diets with peroxidized oil fed to pigs supplemented with or without phytogenics in the water increased protein carbonyls in the serum of pigs in comparison with the control oil treatment and the peroxidized oil with vitamin E. These results suggest that the addition of phytogenics in water did not reduce protein damage from the peroxidized diet. On the other hand, Gerasopoulos *et al*. (2015) analyzing protein carbonyls in piglets fed diets with polyphenols (source of phenols derived from plants) and reported a reduction in plasma protein carbonyls. It seems that phytogenics used in the present study were not efficient to ameliorate protein damage with an oxidative stress challenge induced by feeding dietary peroxidized oil. Lu *et al*. (2014) did not report differences in plasma protein carbonyls when dietary vitamin E was added to peroxidized oil diets for piglets. In the present study, peroxidized oil increased protein carbonyls in serum, which is in agreement with Lindblom *et al*. (2018 and 2019) who found increased levels of plasma protein carbonyls in piglets and broilers, respectively fed peroxidized oil diet. In contrast, Chang *et al*. (2019) did not find effects of peroxidation on protein carbonyl in piglets.

One of the principal compounds formed during lipid peroxidation is malondialdehyde (MDA), which is used to measure cellular lipid peroxidation (Comporti, 1993). In the present study, the levels of MDA were increased with time, suggesting that the presence of lipid peroxidation by oxidative stress was more expressed at the end of the experiment. Moreover, the addition of antioxidants or peroxidized oil did not affect serum MDA. These results are in accordance with those reported by Chang *et al*. (2019) and Lindblom *et al*. (2019) who did not observe effects on MDA in piglets and broilers fed with peroxidized oil. Contrarily, Lindblom *et al*. (2018) observed increased MDA in liver of pigs fed peroxidized oil diets. On the other hand,

Gerasopoulos *et al*. (2015) reported a reduction in plasma MDA in piglets when polyphenols were added to the diet. Likewise, Luehring *et al*. (2011) reported a reduction in MDA when adding quercetin (source of phytogenics) in peroxidized fish oil diets with low intake of vitamin E. Lu *et al*. (2014) did not report differences in plasma MDA when dietary vitamin E was added to peroxidized oil diets for piglets.

Total antioxidant capacity (TAC) is an aggregated action of all antioxidants present in plasma providing a total measurement parameter that can serve as oxidative stress marker (Sies, 2007). In the present study, TAC in serum was increased on d 35 when compared with d 7 of sample collection. This result can be related to the reduction of serum vitamin E with time and the increase in protein carbonyls and MDA, showing reduced antioxidant capacity over time. Additionally, a tendency to decrease TAC was observed with peroxidized oil with or without antioxidant supplementation in comparison with control oil. It seemed that TAC in the body reacted under peroxidation conditions diminishing their values in the serum, but antioxidants were not able to ameliorate this effect. On the other hand, Chang *et al*. (2019) reported a reduction in serum TAC when peroxidation level increased and Gerasopolous *et al*. (2015) found increased values of plasma TAC in piglets using dietary polyphenols. In the present study, the supplementation of vitamin E in water did not affect TAC in the serum of pigs. Likewise Brenes *et al*. (2008) did not report effects on serum TAC in broilers when fed dietary vitamin E (200 mg/kg of α-tocopheryl acetate), but serum TAC was improved when fed 6% of polyphenols in the diet, showing polyphenols in that study had higher antioxidant capacity than vitamin E.

Cytokines are small glycoprotein messengers which have important roles in immune functioning. Cytokines have effects on inflammatory responses (McInnes, 2017). Oxidative stress caused by peroxidation affects intestinal cells. Macrophages migrate to the affected tissues and

produce inflammatory reactions, followed by T cells that promote inflammation in later periods. Pro- and anti-inflammatory cytokines are released during the inflammation process. At the same time, nuclear factor-erythroid 2-related factor-2 (Nrf2) activates expression of antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, to trigger cellular redox homeostasis. Phytogenics can exert effects on Nrf2 to reduce inflammation (Yang *et al*., 2015). In the present study, the majority of cytokines (pro- and anti-inflammatory) were not different due to treatments or day of blood collection. Serum IL-1ra (anti-inflammatory) and IL-8 (proinflammatory) were reduced at the end of the study. Additionally, in this study phytogenics in water did not impact serum TNF-α. Kaschubek *et al*. (2018) and Kroismayr *et al*. (2008) reported significant reductions in TNF-α in intestinal porcine epithelial cells *in vitro* and reduced size of ileal Peyer's patches in piglets, respectively, using essential oils. Also, Deng *et al*. (2010), showed reductions in IL-1 and IFN-γ, alleviating inflammation caused by injected diquat (herbicide which produces superoxide anion radicals and hydrogen peroxide inducing oxidative stress) in piglets using dietary polyphenols.

In conclusion, the inclusion of peroxidized oil in the diet of weaned piglets caused decreased growth performance and this could not be improved by antioxidant supplementation in drinking water. Feed efficiency at week 5 was reduced by peroxidation with or without vitamin E but not with phytogenics treatment. Serum vitamin E increased with supplemental vitamin E, but was not affected by phytogenics. Phytogenic treatment reduced 8-OHdG and protein carbonyls, but increased serum TNF-α. No effects were observed on MDA and peroxidation reduced TAC. The addition of vitamin E and phytogenics in water did not improve growth performance, or oxidative and immune status in weaned piglets fed peroxidized oil diets.

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**References**

AOAC. Official methods of analysis. 15th ed. Arlington (VA): AOAC; 1990

AOCS. Official methods and recommended practices of the American Oil Chemists Society. 5th ed. Champaign (IL): AOCS; 1998

AOAC. Official Methods of Analysis. 18th Edition, Association of Official Analytical Chemists, Gaithersburgs, MD; 2006

Blas, E., Cervera, C., Rodenas, L., Martínez, E., & Pascual, J. J. (2010). The use of recycled oils from the food industry in growing rabbit feeds in substitution of fresh oil does not affect performance. *Animal Feed Science and Technology, 161*(1), 67-74. doi:10.1016/j.anifeedsci.2010.07.011

Boler, D. D., Fernández-Dueñas, D. M., Kutzler, L. W., Zhao, J., Harrell, R. J., Campion, D. R., McKeith, F. K., Killeger, J., & Dilger, A. C. (2012). Effects of oxidized corn oil and a synthetic antioxidant blend on performance, oxidative status of tissues, and fresh meat quality in finishing barrows. *Journal of Animal Science, 90*(13), 5159-5169. doi:10.2527/jas.2012-5266

Botsoglou, N., Christaki, E., Florou-Paneri, P., Giannenas, I., Papageorgiou, G., & Spais, A. (2004). The effect of a mixture of herbal essential oils or á-tocopheryl acetate on performance parameters and oxidation of body lipid in broilers. *South African Journal of Animal Science, 34*(1) doi:10.4314/sajas.v34i1.4039

Brenes, A., Viveros, A., Goñi, I., Centeno, C., Sáyago-Ayerdy, S. G., Arija, I., & Saura-Calixto, F. (2008). Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poultry Science, 87*(2), 307-316. doi:10.3382/ps.2007-00297

Chang, P. L., Boyd, R. D., Zier-Rush, C., Rosero, D. S., & van Heugten, E. (2019). Lipid peroxidation impairs growth and viability of nursery pigs reared under commercial conditions. *Journal of Animal Science,* doi:10.1093/jas/skz183

Choe, E., & Min, D. B. (2007). Chemistry of Deep-Fat frying oils. *Journal of Food Science, 72*(5), R86. doi:10.1111/j.1750-3841.2007.00352.x

Comporti, M. (1993). Lipid peroxidation. biopathological significance. *Molecular Aspects of Medicine, 14*(3), 199-207. doi:10.1016/0098-2997(93)90006-Y

Deng, Q., Xu, J., Yu, B., He, J., Zhang, K., Ding, X., & Chen, D. (2010). Effect of dietary tea polyphenols on growth performance and cell-mediated immune response of post-weaning piglets under oxidative stress. *Archives of Animal Nutrition, 64*(1), 12-21. doi:10.1080/17450390903169138

DeRouchey, J. M., Hancock, J. D., Hines, R. H., Maloney, C. A., Lee, D. J., Cao, H., Dean, D. W., Park, J. S. (2004). Effects of rancidity and free fatty acids in choice white grease on growth performance and nutrient digestibility in weanling pigs. *Journal of Animal Science, 82*(10), 2937-2944. doi:10.2527/2004.82102937x

Gerasopoulos, K., Stagos, D., Petrotos, K., Kokkas, S., Kantas, D., Goulas, P., & Kouretas, D. (2015). Feed supplemented with polyphenolic byproduct from olive mill wastewater processing improves the redox status in blood and tissues of piglets. *Food and Chemical Toxicology, 86*, 319-327. doi:10.1016/j.fct.2015.11.007

Hanson, A. R., Urriola, P. E., Wang, L., Johnston, L. J., Chen, C., & Shurson, G. C. (2016). Dietary peroxidized maize oil affects the growth performance and antioxidant status of nursery pigs. *Animal Feed Science and Technology, 216*, 251-261. doi:10.1016/j.anifeedsci.2016.03.027

Hung, Y. T., Hanson, A. R., Shurson, G. C., & Urriola, P. E. (2017). Peroxidized lipids reduce growth performance of poultry and swine: A meta-analysis. *Animal Feed Science and Technology, 231*, 47-58. doi:10.1016/j.anifeedsci.2017.06.013

Kaschubek, T., Mayer, E., Rzesnik, S., Grenier, B., Bachinger, D., Schieder, C., Konig, J., & Teichmann, K. (2018). Effects of phytogenic feed additives on cellular oxidative stress and inflammatory reactions in intestinal porcine epithelial cells. *Journal of Animal Science, 96*(9), 3657-3669. doi:10.1093/jas/sky263

Kerr, B.J.; Kellner, T.A., & Shurson, G. C. (2015). Characteristics of lipids and their feeding value in swine diets.*Journal of Animal Science and Biotechnology, 6*(1), 30. doi:10.1186/s40104-015-0028-x

Kim, B.G., and M.D. Lindemann. 2007. A spreadsheet method for experimental animal allotment. J. Anim. Sci.85 (Suppl. 2):112.

Kroismayr, A., Sehm, J., Pfaffl, M. W., Schedle, K., Plitzner, C., & Windisch, W. (2008). Effects of avilamycin and essential oils on mRNA expression of apoptotic and inflammatory markers and gut morphology of piglets. *Czech Journal of Animal Science, 53*(No. 9), 377-387. doi:10.17221/338-CJAS

Lindblom, S. C., N. K. Gabler, R. N. Dilger, Z. F. Olson, C. L. Loving, and B. J. Kerr. (2018). Influence of feeding thermally peroxidized soybean oil on oxidative status in growing pigs. *Journal of Animal Science*, 96(2),545–557. doi:10.1093/jas/sky005

Lindblom, S. C., Gabler, N. K., Bobeck, E. A., & Kerr, B. J. (2019). Oil source and peroxidation status interactively affect growth performance and oxidative status in broilers from 4 to 25 d of age. *Poultry Science, 98*(4), 1749. doi: 10.3382/ps/pey547

Liu, P., Chen, C., Kerr, B. J., Weber, T. E., Johnston, L. J., & Shurson, G. C. (2014). Influence of thermally oxidized vegetable oils and animal fats on growth performance, liver gene expression, and liver and serum cholesterol and triglycerides in young pigs1. *Journal of Animal Science, 92*(7), 2960-2970. doi:10.2527/jas.2012-5709

Liu, F., Cottrell, J. J., Furness, J. B., Rivera, L. R., Kelly, F. W., Wijesiriwardana, U., Pustovit, R. V., Fothergill, L. J., Bravo, D. M., Celi, P., Leury, B. J., Gabler, N. K. & Dunshea, F. R. (2016). Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. *Experimental Physiology, 101*(7), 801-810. doi:10.1113/EP085746

Lu, T., Harper, A. F., Zhao, J., Estienne, M. J., & Dalloul, R. A. (2014). Supplementing antioxidants to pigs fed diets high in oxidants: I. effects on growth performance, liver function, and oxidative status1. *Journal of Animal Science, 92*(12), 5455-5463. doi:10.2527/jas.2013-7109

Lúcio, M., Nunes, C., Gaspar, D., Ferreira, H., Lima, J., & Reis, S. (2009). Antioxidant activity of vitamin E and trolox: Understanding of the factors that govern lipid peroxidation studies in vitro. *Food Biophysics, 4*(4), 312-320. doi:10.1007/s11483-009-9129-4

Luehring, M., Blank, R., & Wolffram, S. (2011). Vitamin E-sparing and vitamin E-independent antioxidative effects of the flavonol quercetin in growing pigs. *Animal Feed Science and Technology, 169*(3-4), 199-207. doi:10.1016/j.anifeedsci.2011.06.006

Mateos, R., & Bravo, L. (2007). Chromatographic and electrophoretic methods for the analysis of biomarkers of oxidative damage to macromolecules (DNA, lipids, and proteins). *Journal of Separation Science, 30*(2), 175-191. doi:10.1002/jssc.200600314

McInnes, I. B. (2017). In Firestein G. S., Budd R. C., Gabriel S. E., McInnes I. B. and O'Dell J. R. (Eds.), *Chapter 26 - cytokines* Elsevier. doi://doi.org/10.1016/B978-0-323-31696-5.00026-7

Moeser, A. J., Pohl, C. S., & Rajput, M. (2017). Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. *Animal Nutrition, 3*(4), 313-321. doi:10.1016/j.aninu.2017.06.003

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Panadare, D., Rathod, V. (2015). Applications of waste cooking oil other than biodiesel: A review. *Iranian Journal of Chemical Engineering (IJChE)*, 12(3), 55-76.

Peixoto, E. L. T., Mizubuti, I. Y., Ribeiro, E. L. D., Moura, E. D., Pereira, E. S., do Prado, Odimari, O. P. P., De Carvalho, L. N., & Pires, K. A. (2017). Residual frying oil in the diets of sheep: Intake, digestibility, nitrogen balance and ruminal parameters. *Asian-Australasian Journal of Animal Sciences, 30*(1), 51-56. doi:10.5713/ajas.15.0970

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry, 97*, 55-74. doi:10.1016/j.ejmech.2015.04.040

Rooke, J. A., Robinson, J. J., & Arthur, J. R. (2004). Effects of vitamin E and selenium on the performance and immune status of ewes and lambs. *The Journal of Agricultural Science, 142*(3), 253-262. doi:10.1017/S0021859604004368

Rosero, D. S., Odle, J., Moeser, A. J., Boyd, R. D., & van Heugten, E. (2015). Peroxidised dietary lipids impair intestinal function and morphology of the small intestine villi of nursery pigs in a dose-dependent manner. *The British Journal of Nutrition, 114*(12), 1985-1992. doi:10.1017/S000711451500392X

Santos, C. S. P., Molina-Garcia, L., Cunha, S. C., & Casal, S. (2018). Fried potatoes: Impact of prolonged frying in monounsaturated oils. *Food Chemistry, 243*, 192-201. doi:10.1016/j.foodchem.2017.09.117

Schöne, F., Vetter, A., Hartung, H., Bergmann, H., Biertümpfel, A., Richter, G., Muller, S. & Breitschuh, G. (2006). Effects of essential oils from fennel (foeniculi aetheroleum) and caraway (carvi aetheroleum) in pigs. *Journal of Animal Physiology and Animal Nutrition, 90*(11-12), 500-510. doi:10.1111/j.1439-0396.2006.00632.x

Sies, H. (2007). Total antioxidant capacity: Appraisal of a Concept. *The Journal of Nutrition, 137*(6), 1493. doi:10.1093/jn/137.6.1493

Sies, H., Stahl, W., & Sundquist, A. R. (1992). Antioxidant functions of vitamins. *Annals of the New York Academy of Sciences, 669*(1), 7-20. doi:10.1111/j.1749-6632.1992.tb17085.x

Singh-Ackbarali, D., Maharaj, R., Mohamed, N., & Ramjattan-Harry, V. (2017). Potential of used frying oil in paving material: Solution to environmental pollution problem. *Environmental Science and Pollution Research International, 24*(13), 12220-12226. doi:10.1007/s11356-017-8793-z

Shurson, G. C., Kerr, B. J., & Hanson, A. R. (2015). Evaluating the quality of feed fats and oils and their effects on pig growth performance. *Journal of Animal Science and Biotechnology, 6*(1), 10. doi:10.1186/s40104-015-0005-4

van Heugten, E., Sweet, L.A., Stumpf, T.T., Risley, C.R. and Schell, T.C. 1997. Effects of water supplementation with selenium and vitamin E on growth performance and blood selenium and serum vitamin E concentrations in weanling pigs. J. Am. Vet. Med. Assoc. 211:1039-1042.

Windisch, W., Schedle, K., Plitzner, C., & Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine and poultry1. *Journal of Animal Science, 86*(suppl_14), E148. doi:10.2527/jas.2007-0459

Yang, C., Chowdhury, M. A. K., Huo, Y., & Gong, J. (2015). Phytogenic compounds as alternatives to in-feed antibiotics: Potentials and challenges in application. *Pathogens (Basel, Switzerland), 4*(1), 137-156. doi:10.3390/pathogens4010137

Zhang, T., Zhou, Y. F., Zou, Y., Hu, X. M., Zheng, L. F., Wei, H. K., Giannenas, I., Jin, L.Z., Peng, J. & Jiang, S. W. (2015). Effects of dietary oregano essential oil supplementation on the stress response, antioxidative capacity, and HSPs mRNA expression of transported pigs. *Livestock Science, 180*, 143-149. doi:10.1016/j.livsci.2015.05.037

**Table 1.** Composition of the experimental Phase 1 diets. As fed basis[1]

| | Treatments | | | |
|---|---|---|---|---|
| | Control oil | Peroxidized oil | | |
| Item | **No antioxidant** | **No antioxidant** | **With vitamin E** | **With phytogenics** |
| **Ingredient, %** | | | | |
| Corn, yellow dent | 36.35 | 36.35 | 36.35 | 36.35 |
| Soybean meal, 47.5% CP | 21.00 | 21.00 | 21.00 | 21.00 |
| Whey permeate | 20.00 | 20.00 | 20.00 | 20.00 |
| Poultry byproduct meal | 6.76 | 6.76 | 6.76 | 6.76 |
| Fish meal, menhaden | 4.00 | 4.00 | 4.00 | 4.00 |
| Plasma spray-dried | 4.00 | 4.00 | 4.00 | 4.00 |
| Soybean oil[2] | 6.00 | 6.00 | 6.00 | 6.00 |
| Monocalcium phosphate 21% | 0.35 | 0.35 | 0.35 | 0.35 |
| Limestone | 0.24 | 0.24 | 0.24 | 0.24 |
| Zinc oxide, 72% Zn | 0.26 | 0.26 | 0.26 | 0.26 |
| Copper sulfate, 25.2% Cu | 0.07 | 0.07 | 0.07 | 0.07 |
| L-lysine HCl 78.5% | 0.27 | 0.27 | 0.27 | 0.27 |
| DL-methionine | 0.20 | 0.20 | 0.20 | 0.20 |
| L-threonine | 0.13 | 0.13 | 0.13 | 0.13 |
| L-tryptophan | 0.01 | 0.01 | 0.01 | 0.01 |
| Salt | 0.15 | 0.15 | 0.15 | 0.15 |
| Mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 |
| Vitamin premix[4] | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamin E[5], IU/L[5] | - | - | 100 | - |
| Phytogenic compounds, µL/L | - | - | - | 60 or 30 |
| **Calculated Composition** | | | | |
| ME, kcal/kg | 3609 | 3609 | 3609 | 3609 |
| Crude Protein % | 24.20 | 24.20 | 24.20 | 24.20 |
| Lactose % | 16.00 | 16.00 | 16.00 | 16.00 |
| SID Amino acids | | | | |
| Lys % | 1.50 | 1.50 | 1.50 | 1.50 |
| Thr % | 0.93 | 0.93 | 0.93 | 0.93 |
| Met % | 0.53 | 0.53 | 0.53 | 0.53 |
| Met+Cys% | 0.87 | 0.87 | 0.87 | 0.87 |
| Trp % | 0.27 | 0.27 | 0.27 | 0.27 |
| Total Lysine % | 1.70 | 1.70 | 1.70 | 1.70 |
| **Analyzed composition[6]** | | | | |
| Crude protein, % | 22.90 | 23.87 | 23.22 | 23.22 |
| Moisture, % | 8.95 | 8.89 | 8.68 | 8.68 |
| Crude fat, % | 7.22 | 7.20 | 7.64 | 7.64 |
| Crude fiber, % | 1.71 | 1.77 | 1.69 | 1.69 |
| Ash, % | 6.26 | 6.37 | 6.35 | 6.35 |
| Ca, % | 0.77 | 0.88 | 0.91 | 0.91 |
| P, % | 0.76 | 0.79 | 0.80 | 0.80 |

**Table 1.** Continued

[1] Diets were formulated to exceed NRC (2012) requirements. Phase 1 diets were fed from d 0 to d 14

[2] Control or peroxidized soybean oil. Peroxidation was created by heating control oil at 80ºC while bubbling air through oil at the rate of 50L/min for 12 d. Control and peroxidized soybean oil were stabilized with 0.1% liquid antioxidant tertiary butyl hydroquinone (TBHQ) after peroxidation

[3] Supplied per kg of complete diet: 33 mg of Mn, 110 mg of Zn, 110 mg of Fe, 17 mg of Cu, 0.30 mg of I and 0.30 mg of Se

[4] Supplied per kg of complete diet: 9700 IU of vitamin A, 871 IU of vitamin $D_3$, 66.1 IU of vitamin E, 0.03 mg of vitamin $B_{12}$ as d-calcium pantothenate, 3.6 mg of vitamin K as menadione sodium bisulfite complex, 11 mg of riboflavin, 27.6 mg of d-pantothenic acid, 44.1 mg of niacin, 2.5 mg of vitamin $B_6$ as pyridoxine hydrochloride, 2.6 mg of folic acid and 0.44 mg of biotin.

[5] Emcelle®, tocopherol, Stuart products, Bedford Road, Bedford, TX. Added to drinking water during the entire experiment

[6] Analyzed by Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO

**Table 2.** Composition of the experimental Phase 2 diets. As fed basis[1]

| | Treatments | | | |
|---|---|---|---|---|
| | Control oil | Peroxidized oil | | |
| Item | **No antioxidant** | **No antioxidant** | **With vitamin E** | **With phytogenics** |
| **Ingredient, %** | | | | |
| Corn, yellow dent | 55.34 | 55.34 | 55.34 | 55.34 |
| Soybean meal, 47.5% CP | 34.67 | 34.67 | 34.67 | 34.67 |
| Soybean oil[2] | 6.00 | 6.00 | 6.00 | 6.00 |
| Monocalcium phosphate 21% | 1.53 | 1.53 | 1.53 | 1.53 |
| Limestone | 1.16 | 1.16 | 1.16 | 1.16 |
| Copper sulfate, 25.2% Cu | 0.07 | 0.07 | 0.07 | 0.07 |
| L-lysine HCl 78.5% | 0.36 | 0.36 | 0.36 | 0.36 |
| DL-methionine | 0.18 | 0.18 | 0.18 | 0.18 |
| L-threonine | 0.14 | 0.14 | 0.14 | 0.14 |
| Salt | 0.35 | 0.35 | 0.35 | 0.35 |
| Mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 |
| Vitamin premix[4] | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamin E[5], IU/L | - | - | 100 | - |
| Phytogenic compounds, µL/L | - | - | - | 60 or 30 |
| **Calculated Composition** | | | | |
| ME, kcal/kg | 3556 | 3556 | 3556 | 3556 |
| Crude Protein % | 21.70 | 21.70 | 21.70 | 21.70 |
| SID Amino acids | | | | |
| Lys % | 1.30 | 1.30 | 1.30 | 1.30 |
| Thr % | 0.81 | 0.81 | 0.81 | 0.81 |
| Met % | 0.47 | 0.47 | 0.47 | 0.47 |
| Met+Cys% | 0.75 | 0.75 | 0.75 | 0.75 |
| Trp % | 0.23 | 0.23 | 0.23 | 0.23 |
| Total Lysine % | 1.45 | 1.45 | 1.45 | 1.45 |
| **Analyzed composition[6]** | | | | |
| Crude protein, % | 20.48 | 20.48 | 20.48 | 20.48 |
| Moisture, % | 11.68 | 11.68 | 11.68 | 11.68 |
| Crude fat, % | 4.67 | 4.67 | 4.67 | 4.67 |
| Crude fiber, % | 2.27 | 2.27 | 2.27 | 2.27 |
| Ash, % | 5.09 | 5.09 | 5.09 | 5.09 |
| Ca, % | 0.79 | 0.79 | 0.79 | 0.79 |
| P, % | 0.67 | 0.67 | 0.67 | 0.67 |

[1]Diets were formulated to exceed NRC (2012) requirements. Phase 2 diets were fed from d 15 to d 35

[2] Control or peroxidized soybean oil. Peroxidation was created by heating control oil at 80ºC while bubbling air through oil at the rate of 50L/min for 12 d. control and peroxidized soybean oils were stabilized 0.1% liquid antioxidant tertiary butyl hydroquinone (TBHQ) after peroxidation

[3] Supplied per kg of complete diet: 33 mg of Mn, 110 mg of Zn, 110 mg of Fe, 17 mg of Cu, 0.30 mg of I and 0.30 mg of Se

**Table 2.** Continued

[4]Supplied per kg of complete diet: 9700 IU of vitamin A, 871 IU of vitamin $D_3$, 66.1 IU of vitamin E, 0.03 mg of vitamin $B_{12}$ as d-calcium pantothenate, 3.6 mg of vitamin K as menadione sodium bisulfite complex, 11 mg of riboflavin, 27.6 mg of d-pantothenic acid, 44.1 mg of niacin, 2.5 mg of vitamin $B_6$ as pyridoxine hydrochloride, 2.6 mg of folic acid and 0.44 mg of biotin.

[5] Emcelle®, tocopherol, Stuart products, Bedford Road, Bedford, TX. Added to drinking water during whole experiment

[6] Analyzed by Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO

**Table 3.** Composition of experimental soybean oils[1]

| Item | **Control** | **Peroxidized** |
|---|---|---|
| Moisture, % | 0.10 | 0.11 |
| Insoluble impurities, % | 0.06 | none |
| Unsaponifiable matter, % | 0.38 | 0.34 |
| Free fatty acids, % | 0.02 | 0.09 |
| Peroxide Value, mEq/kg fat | | |
| Initial[2] | 4.1 | 141.6 |
| 4hr AOM[3] | 3.6 | 158 |
| 20hr AOM | 4.6 | 41.2 |
| Anisidine value | 1.7 | 106 |
| Oxidative Stability Index (OSI), hours[4] | 37 | 2.4 |
| Hexanal, ppm[4] | <5 | 99 |
| 2, 4-Decadienal, ppm[4] | 8 | 720 |
| Antioxidant, % | 0.1 | 0.1 |

[1]Analyzed by New Jersey Feed Laboratory Inc., Trenton, NJ
[2]Analyzed by Kemin Industries, Inc. Des Moines, IA and New Jersey Feed Laboratory Inc., Trenton, NJ, both analyzed initial peroxide value and the mean is reported here.
[3]AOM: Active oxygen method
[4]Analyzed by Kemin Industries, Inc. Des Moines, IA

**Table 4.** Growth performance of weaned pigs fed antioxidants in drinking water when fed peroxidized diets during a 35-day experimental period[1]

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | Control oil | Peroxidized oil | | | | *P* - value |
| Item | No antioxidant | No antioxidant | With vitamin E | With phytogenics | SEM | Treatment |
| Body weight, kg | | | | | | |
| d0 | 6.11 | 6.08 | 6.09 | 6.10 | 0.281 | 0.573 |
| d7 | 6.94 | 6.97 | 7.18 | 6.99 | 0.290 | 0.303 |
| d14 | 9.50 | 9.44 | 9.55 | 9.41 | 0.437 | 0.980 |
| d21 | 12.4 | 11.8 | 12.0 | 12.0 | 0.538 | 0.613 |
| d28 | 16.5a | 14.8b | 14.6b | 14.8b | 0.650 | 0.010 |
| d35 | 21.6a | 18.2b | 17.8b | 18.7b | 0.775 | <0.001 |
| ADG, kg | | | | | | |
| d 0 to 7 | 0.119 | 0.126 | 0.157 | 0.128 | 0.015 | 0.254 |
| d 7 to 14 | 0.366 | 0.354 | 0.338 | 0.346 | 0.027 | 0.884 |
| d 14 to 21 | 0.414a | 0.327b | 0.345ab | 0.373ab | 0.029 | 0.030 |
| d 21 to 28 | 0.586a | 0.431b | 0.369b | 0.400b | 0.030 | <0.001 |
| d 28 to 35 | 0.722a | 0.493b | 0.465b | 0.543b | 0.035 | <0.001 |
| ADG, kg (phases) | | | | | | |
| Phase 1 | 0.242 | 0.240 | 0.248 | 0.237 | 0.018 | 0.978 |
| Phase 2 | 0.574a | 0.417b | 0.393b | 0.439b | 0.023 | <0.001 |
| Overall ADG | 0.441a | 0.346b | 0.335b | 0.357b | 0.016 | <0.001 |
| ADFI, kg | | | | | | |
| d 0 to 7 | 0.162 | 0.179 | 0.202 | 0.180 | 0.016 | 0.396 |
| d 7 to 14 | 0.437 | 0.403 | 0.417 | 0.427 | 0.021 | 0.700 |
| d 14 to 21 | 0.621a | 0.531b | 0.547ab | 0.532b | 0.027 | 0.048 |
| d 21 to 28 | 0.83a | 0.70ab | 0.68ab | 0.65b | 0.053 | 0.044 |
| d 28 to 35 | 1.03 | 0.89 | 0.86 | 0.85 | 0.066 | 0.241 |

**Table 4.** Continued

| | | | | | | |
|---|---|---|---|---|---|---|
| ADFI, kg (phases) | | | | | | |
| Phase 1 | 0.306 | 0.291 | 0.310 | 0.294 | 0.017 | 0.818 |
| Phase 2 | 0.824 | 0.709 | 0.697 | 0.674 | 0.043 | 0.061 |
| Overall ADFI | 0.617 | 0.542 | 0.542 | 0.527 | 0.030 | 0.136 |
| G: F | | | | | | |
| d 0 to 7 | 0.732 | 0.696 | 0.768 | 0.701 | 0.038 | 0.487 |
| d 7 to 14 | 0.841 | 0.872 | 0.805 | 0.806 | 0.031 | 0.325 |
| d 14 to 21 | 0.666 | 0.618 | 0.632 | 0.690 | 0.038 | 0.140 |
| d 21 to 28 | 0.710a | 0.625ab | 0.550b | 0.621ab | 0.035 | 0.033 |
| d 28 to 35 | 0.703a | 0.562b | 0.544b | 0.650ab | 0.032 | 0.007 |
| G: F (phases) | | | | | | |
| Phase 1 | 0.792 | 0.820 | 0.793 | 0.808 | 0.028 | 0.796 |
| Phase 2 | 0.696a | 0.597b | 0.569b | 0.651ab | 0.023 | 0.004 |
| Overall G: F | 0.715a | 0.645ab | 0.621b | 0.678ab | 0.018 | 0.012 |

[1]Values represent least squares means of 6 pens with 4 pigs (2 gilts and 2 barrows) per pen
[ab] means within a row without a common superscript are significantly different ($P < 0.05$)

**Table 5.** Serum vitamin E concentration in newly weaned pigs fed diets with or without peroxidized oil and supplemented with or without antioxidants in the drinking water[1]

| | | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control oil | Peroxidized oil | | | | *P* - value | | |
| Item | Day | No antioxidant | No antioxidant | With vitamin E | With phytogenics | SEM | Day | Treatment | Day x Treatment |
| Vitamin E, ppm | | | | | | | | | |
| | 7 | 1.88 | 1.27 | 1.72 | 1.40 | 0.284 | 0.012 | 0.018 | 0.643 |
| | 35 | 1.15 | 0.57 | 1.60 | 0.55 | | | | |

[1]Values represent least squares means of 6 pigs (equally representing gilts and barrows)

**Table 6.** Oxidative stress and antioxidant capacity of newly weaned piglets fed diets with or without peroxidized oil supplemented with or without antioxidants in the drinking water[1]

| | | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control oil | Peroxidized oil | | | | *P* - value | | |
| Item | Day | No antioxidant | No antioxidant | With vitamin E | With phytogenics | SEM | Day | Treatment | Day x Treatment |
| 8-OHdG[2], ng/mL | | | | | | | | | |
| | 7 | 3.95 | 2.71 | 3.81 | 2.99 | 0.354 | 0.202 | 0.007 | 0.200 |
| | 35 | 3.91 | 2.68 | 2.51 | 3.04 | | | | |
| Pt Carbonyl, pmol/mg protein | | | | | | | | | |
| | 7 | 2.36 | 1.91 | 2.66 | 1.93 | 0.370 | 0.011 | 0.564 | 0.029 |
| | 35 | 1.95 | 3.24 | 3.04 | 2.81 | | | | |
| MDA[3], µM | | | | | | | | | |
| | 7 | 11.9 | 9.2 | 8.4 | 9.8 | 1.200 | 0.003 | 0.339 | 0.303 |
| | 35 | 11.9 | 13.4 | 11.7 | 13.6 | | | | |
| TAC[4], CRE[5] | | | | | | | | | |
| | 7 | 281 | 254 | 262 | 256 | 16.358 | <0.001 | 0.099 | 0.341 |
| | 35 | 357 | 338 | 291 | 329 | | | | |

[1]Values represent least squares means of 6 pigs (equally representing gilts and barrows)
[2]8-OHdG: 8-hydroxydeoxyguanosine
[3]MDA: malondialdehyde
[4]TAC: total antioxidant capacity
[5]CRE: copper reducing equivalent

**Table 7.** Immune stress status of newly weaned piglets fed diets with or without peroxidized oil supplemented with or without antioxidants in the drinking water[1]

| | | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control oil | Peroxidized oil | | | | *P* - value | | |
| Item | Day | No antioxidant | No antioxidant | With vitamin E | With phytogenics | SEM | Day | Treatment | Day x Treatment |
| IFN-γ[2], pg/mL | | | | | | | | | |
| | 7 | 8890 | 9650 | 11079 | 15630 | 3411.23 | 0.244 | 0.275 | 0.900 |
| | 35 | 12774 | 11904 | 17063 | 16186 | | | | |
| IL-1α[3], pg/mL | | | | | | | | | |
| | 7 | 59 | 50 | 51 | 44 | 27.371 | 0.688 | 0.886 | 0.840 |
| | 35 | 50 | 80 | 39 | 67 | | | | |
| IL-1β[4], pg/mL | | | | | | | | | |
| | 7 | 473 | 430 | 526 | 448 | 185.27 | 0.746 | 0.764 | 0.856 |
| | 35 | 498 | 272 | 335 | 577 | | | | |
| IL-1ra[5], pg/mL | | | | | | | | | |
| | 7 | 1752 | 1602 | 1425 | 1280 | 330.76 | 0.015 | 0.79 | 0.671 |
| | 35 | 685 | 859 | 550 | 1109 | | | | |
| IL-2[6], pg/mL | | | | | | | | | |
| | 7 | 413 | 331 | 353 | 351 | 222.05 | 0.791 | 0.915 | 0.876 |
| | 35 | 356 | 589 | 266 | 416 | | | | |
| IL-4[7], pg/mL | | | | | | | | | |
| | 7 | 906 | 1624 | 1671 | 1468 | 1022.06 | 0.39 | 0.61 | 0.298 |
| | 35 | 3992 | 931 | 991 | 2500 | | | | |
| IL-6[8], pg/mL | | | | | | | | | |
| | 7 | 63 | 54 | 92 | 76 | 28.4 | 0.367 | 0.55 | 0.811 |
| | 35 | 58 | 26 | 37 | 78 | | | | |
| IL-8[9], pg/mL | | | | | | | | | |
| | 7 | 903 | 754 | 589 | 846 | 158.94 | 0.003 | 0.329 | 0.805 |
| | 35 | 686 | 381 | 326 | 384 | | | | |

**Table 7**. Continued

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| IL-10[10], pg/mL | | | | | | | | |
| | 7 | 1002 | 747 | 1001 | 1194 | 416.91 | 0.606 | 0.688 | 0.999 |
| | 35 | 870 | 562 | 770 | 1054 | | | | |
| IL-12[11], pg/mL | | | | | | | | |
| | 7 | 1205 | 1036 | 1063 | 843 | 169.78 | 0.516 | 0.166 | 0.764 |
| | 35 | 1159 | 1030 | 729 | 874 | | | | |
| IL-18[12], pg/mL | | | | | | | | |
| | 7 | 730 | 1538 | 1566 | 1549 | 396.47 | 0.492 | 0.647 | 0.570 |
| | 35 | 1212 | 958 | 943 | 1404 | | | | |
| TNF-α[13], pg/mL | | | | | | | | |
| | 7 | 294 | 274 | 64 | 790 | 166.85 | 0.333 | 0.068 | 0.284 |
| | 35 | 368 | 142 | 164 | 226 | | | | |

[1]Values represent least squares means of 6 pigs (equally representing gilts and barrows)
[2] IFN-γ: interferon-gamma
[3] IL-α: interleukin-alpha
[4] IL-β: interleukin-beta
[5] IL-1ra: interleukin-1receptor antagonist
[6] IL-2: interleukin-2
[7] IL-4: interleukin-4
[8] IL-6: interleukin-6
[9] IL-8: interleukin-8
[10] IL-10: interleukin-10
[11] IL-12: interleukin-12
[12] IL-18: interleukin-18
[13] TNF-α: tumor necrosis factor- alpha

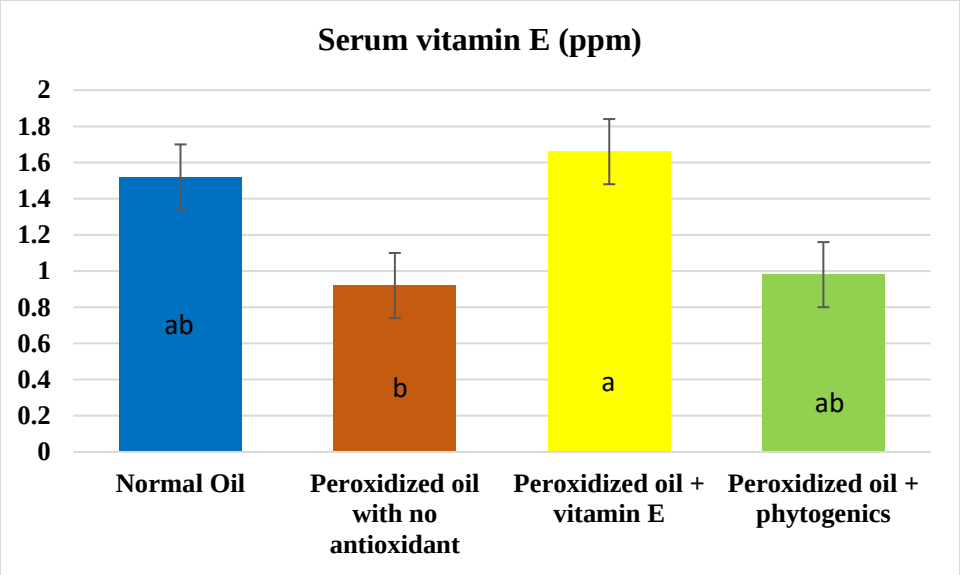


**Figure 1.** Concentration of vitamin E in serum of newly weaned piglets fed diets with or without peroxidized oil supplemented with or without antioxidants in the drinking water. Bars with different letters in the figure are statistical different ($P < 0.05$).

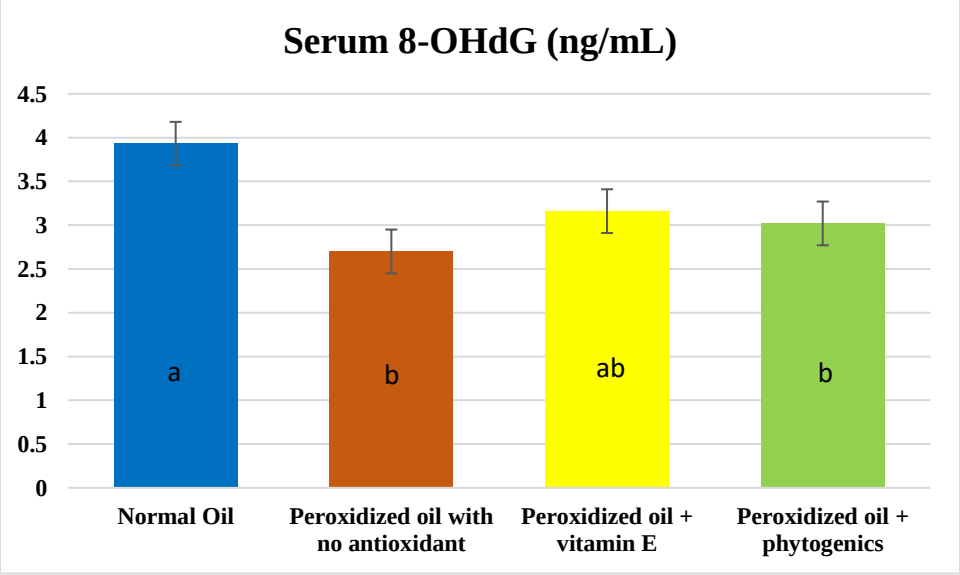


**Figure 2.** Concentration of 8-dihydroxydiguanosin in serum of newly weaned piglets fed diets with or without peroxidized oil supplemented with or without antioxidants. Bars with different letters are statistically different.

# CHAPTER V:

## General discussion

### Introduction

Heat stress is caused in pigs by the exposure to high environmental temperatures causing negative impacts on productivity (St-Pierre *et al*., 2003). Similarly, the addition of peroxidized oils in the feed for pigs causes excessive production of free radicals in cells causing damage to cellular components. Both heat stress and dietary peroxidized oil cause an imbalance between oxidants and the natural antioxidant system, compromising performance and health in pigs. The studies presented in this dissertation evaluated the use of different types of antioxidants in the diet or drinking water as strategies to improve the imbalance between oxidants and antioxidants, and subsequently growth performance, when using high ambient temperatures or peroxidized dietary oil to cause oxidative stress. The stress models used in these studies caused significant reductions in growth performance of pigs (Table 1), providing an opportunity to determine whether nutritional abatement interventions could be successful.

**Table 1.** Percent reduction in growth performance associated with heat stress and dietary peroxidized lipids

| Item | Heat stress (Chapter 2) | Peroxidized oil (Chapter 3) | Peroxidized oil (Chapter 4) |
|---|---|---|---|
| ADG | 26.7 | 26.8 | 21.5 |
| ADFI | 21.9 | 18.7 | 12.2 |
| G:F | 6.3 | 10.0 | 9.8 |

The use of vitamin E, polyphenols and phytogenics in feed or drinking water to alleviate oxidative stress conditions in pigs has only been evaluated in a few studies (Luehring *et al*., 2011; Boler *et al*., 2012; Lu *et al*., 2014; Gerasopoulos *et al*., 2015; and Lindblom *et al*., 2018). In the present dissertation, we studied the addition of vitamin E, polyphenols and phytogenics in the diet

and in the drinking water as supplementation strategies to improve growth performance and oxidative stress through the measurement of antioxidant levels in the body, oxidative stress markers, and immune status. In general, the results showed that the addition of these antioxidants in the feed or in drinking water did not improve growth performance and immune status, but increased levels of antioxidants in serum when using sources of vitamin E, but not polyphenol or phytogenics.

**Effects of vitamin E and polyphenols as antioxidants in feed and in drinking water in growing pigs housed under heat stress**

In Chapter I, the impact of heat stress and oxidative stress caused by feeding peroxidized oil was reviewed and the potential benefits of using vitamin E, polyphenols and phytogenics as antioxidants in the feed and in drinking water in growing pigs was summarized. In Chapter II, the effects of vitamin E and polyphenols in the feed and in drinking water for growing pigs housed under heat stress were evaluated. Even though, the addition of the vitamin E and polyphenols in the feed or in drinking water did not improve growth performance of the pigs housed under heat stress or thermo-neutral conditions, the addition of the natural source of vitamin E in the drinking water and the dietary synthetic source of vitamin E increased serum and liver concentrations of vitamin E, regardless of environmental temperature. The addition of vitamin E in the feed increased the villus to crypt ratio in the jejunum. Likewise, cellular proliferation of enterocytes was increased when using the natural and synthetic sources of vitamin E when the pigs were exposed to heat stress. These results showed that the addition of vitamin E can improve the histology and the proliferation of the intestine.

Immune status measured in serum was not affected by heat stress, but the concentration of tumor necrosis factor-α in the jejunum and in the ileum was decreased by heat stress. Moreover,

in the ileum, the addition of vitamin E in the drinking water decreased tumor necrosis factor-α, but no effects were detected due to supplementation of polyphenols. These results suggest that the addition of vitamin E can reduce inflammation in the intestine in comparison with the polyphenol treatment. According to Grotto *et al*. (2009), heat stress causes peroxidation of lipids of cell membranes, measured by malondialdehyde in serum or intestinal tissue. However, in our experiment, high concentrations of malondialdehyde were only observed following long-term exposure to high heat. Moreover, lipid peroxidation (MDA) was increased when using dietary vitamin E and polyphenols during heat stress in the ileum, but not when using vitamin E in the drinking water. These results showed that heat stress caused lipid peroxidation in pigs when measured over time and the addition of the vitamin E in the water was a more effective way to reduce that lipid peroxidation in the ileum tissue. Thus, the natural source of the vitamin E could have been effective to counteract lipid peroxidation in pigs.

**Effects of dietary vitamin E and polyphenol compounds for weaned piglets fed peroxidized oil diets**

In Chapter III, the effects of supplementation of synthetic vitamin E (dl-α-tpcopheryl acetate) and polyphenol compounds in peroxidized oil diets fed to pigs was determined. Feed efficiency was decreased during the first week of the study by the addition of vitamin E when using peroxidized oil, even though serum vitamin E concentrations were increased. van Heugten *et al*. (1997) showed that the addition of vitamin E combined with selenium in the drinking water increased feed efficiency during their 5-week study. In general terms, it seems that the addition of selenium in combination with vitamin E in the drinking water creates a synergy, thus improving feed efficiency in piglets. Perhaps the addition of other antioxidants in combination with vitamin E can be improve performance and health of pigs fed peroxidized lipids.

In terms of oxidative status, protein carbonyls were increased with the addition of polyphenols in the diet. This result was unexpected. According to Zhang and Tsao (2016), polyphenols are considered as one of the most important groups of antioxidants with antioxidants properties. Although, polyphenols can also have pro-oxidant properties such as chemical instability, variation in glutathione enzyme and mobilization of copper ions into cells (Surai, 2014). Seemingly in this study, the addition of polyphenols in the diet may have displayed pro-oxidant effects to cellular proteins, causing increased protein carbonyls in the serum. This effect was not observed for vitamin E. Lipid peroxidation measured by malondialdehyde was decreased in serum when piglets were fed peroxidized oil. Peroxidation effects may not have manifested themselves systemically when measured in serum, but we can speculate that local cellular lipid peroxidation could occur in intestinal tissue. Rosero *et al*. (2015) reported increased malondialdehyde in jejunal mucosa samples when peroxidized oil was fed. When total antioxidant capacity (TAC) was measured in serum, the addition of vitamin E or polyphenols reduced TAC when pigs were fed peroxidized oil diets. This response would correspond to the capacity of the vitamin E and polyphenols to interact with free radicals, reducing their levels in serum.

Immune status determined by the analysis of serum cytokines showed increased concentrations of cytokines when pigs were fed peroxidized oil for 35 days, but the addition of the vitamin E or polyphenols in the diet did not affect pro- and anti-inflammatory cytokines.

**Effects of vitamin E and phytogenics in the drinking water for weaned piglets fed peroxidized oil diets**

In Chapter IV, the effects of the addition of natural vitamin E or phytogenics in the drinking water for piglets fed peroxidized oil diets was determined. When using natural vitamin E via drinking water of piglets, feed efficiency was reduced during week 4 and overall. Serum

concentrations of vitamin E were higher when pigs consumed drinking water with supplemental vitamin E. Interestingly, a similar response in feed efficiency was detected when vitamin E was added in the feed (Chapter 3). No effects on performance or serum vitamin E were found when phytogenics were added in drinking water. Thus, phytogenics as an antioxidant additive in drinking water did not exert effects on the regeneration or sparing of vitamin E. Interestingly, the addition of phytogenics decreased cellular DNA damage as measured by 8-hydroxydeoxyguanosine (8-OHdG) in serum. This suggests that active components of phytogenics had greater protective effects on cellular DNA than other cellular components.

The use of antioxidants such as vitamin E in feed or drinking water and polyphenols were not effective in alleviating the negative impacts of heat stress or oxidative stress in young pigs. Further research is needed and may include the investigation of different sources and combinations of dietary antioxidants in feed and drinking water to reduce the negative impact of heat stress in growing pigs and oxidative stress in weaned piglets fed peroxidized oils. This could include a mixture of fat-soluble and water-soluble antioxidants.

**References**

Boler, D. D., Fernández-Dueñas, D. M., Kutzler, L. W., Zhao, J., Harrell, R. J., Campion, D. R., McKeith, F. K., Killeger, J., & Dilger, A. C. (2012). Effects of oxidized corn oil and a synthetic antioxidant blend on performance, oxidative status of tissues, and fresh meat quality in finishing barrows. *Journal of Animal Science, 90*(13), 5159-5169. doi:10.2527/jas.2012-5266

Gerasopoulos, K., Stagos, D., Petrotos, K., Kokkas, S., Kantas, D., Goulas, P., & Kouretas, D. (2015). Feed supplemented with polyphenolic byproduct from olive mill wastewater processing improves the redox status in blood and tissues of piglets. doi://doi.org/10.1016/j.fct.2015.11.007

Grotto, D., Maria, L. S., Valentini, J., Paniz, C., Schmitt, G., Garcia, S. C., Pomblum, V. J., Rocha, J. B. T., & Farina, M. (2009). Importance of the lipid peroxidation biomarkers and methodological aspects FOR malondialdehyde quantification. *Química Nova*, *32*(1), 169-174. doi:10.1590/S0100-40422009000100032

Lindblom, S. C., N. K. Gabler, R. N. Dilger, Z. F. Olson, C. L. Loving, and B. J. Kerr. (2018). Influence of feeding thermally peroxidized soybean oil on oxidative status in growing pigs. *Journal of Animal Science*, 96(2),545–557. doi:10.1093/jas/sky005

Lu, T., Harper, A. F., Zhao, J., Estienne, M. J., & Dalloul, R. A. (2014). Supplementing antioxidants to pigs fed diets high in oxidants: I. effects on growth performance, liver function, and oxidative status. *Journal of Animal Science, 92*(12), 5455-5463. doi:10.2527/jas.2013-7109

Luehring, M., Blank, R., & Wolffram, S. (2011). Vitamin E-sparing and vitamin E-independent antioxidative effects of the flavonol quercetin in growing pigs. *Animal Feed Science and Technology, 169*(3-4), 199-207. doi:10.1016/j.anifeedsci.2011.06.006

Rosero, D. S., Odle, J., Moeser, A. J., Boyd, R. D., & van Heugten, E. (2015). Peroxidised dietary lipids impair intestinal function and morphology of the small intestine villi of nursery pigs in a dose-dependent manner. *The British Journal of Nutrition, 114*(12), 1985-1992. doi:10.1017/S000711451500392X

St-Pierre, N. R., Cobanov, B., & Schnitkey, G. (2003). Economic losses from heat stress by US livestock industries. *Journal of Dairy Science, 86*(13 Electronic Supplement 1), E77. doi:10.3168/jds.S0022-0302(03)74040-5

Surai, P. F. (2014). Polyphenol compounds in the chicken/animal diet: From the past to the future. *Journal of Animal Physiology and Animal Nutrition, 98*(1), 19-31. doi:10.1111/jpn.12070

van Heugten, E., Sweet, L.A., Stumpf, T.T., Risley, C.R. and Schell, T.C. 1997. Effects of water supplementation with selenium and vitamin E on growth performance and blood selenium and serum vitamin E concentrations in weanling pigs. J. Am. Vet. Med. Assoc. 211:1039-1042.

Zhang, H., & Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science, 8*, 33-42. doi:10.1016/j.cofs.2016.02.002