20th Annual Midwest Swine Nutrition Conference Proceedings

Danville, Indiana—September 9, 2021

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Midwest Swine Nutrition Conference

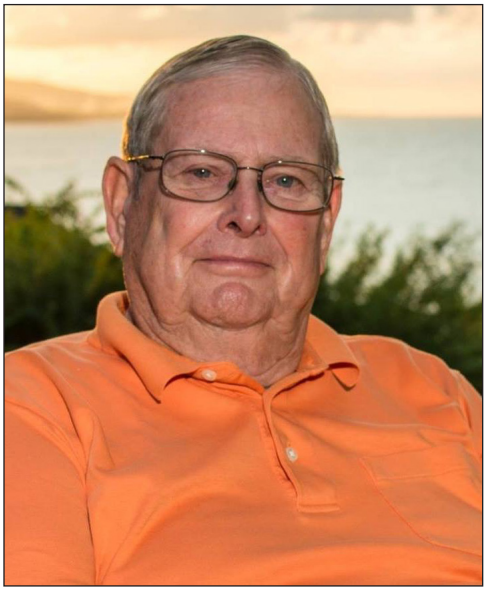
Schedule of Presentations

8:15	Registration
9:00	Welcome *Dennis Liptrap, Ralco Nutrition*
9:05	Aspects of Sow Nutrition during Transition and Lactation to Improve Their Productivity and Feed Efficiency *Peter Theil, Aarhus University, Denmark*
9:50	Thermo-Regulation in Neo-natal Piglets—Implications for Survival *Mike Ellis, University of Illinois*
10:25	Break
11:00	The Effect of Different Fat Supplements and Multiple Levels of Vitamin E on Performance and Meat Quality of Pigs Taken to 150 Kg Market Weight *Merlin Lindemann, University of Kentucky*
11:30	The Effect of Feeding High Oleic Soy Oil in Combination with DDGS on Meat Quality *Bailey Harsh, University of Illinois*
12:00	Lunch
1:00	Nutritional Impact on Mammary Development in Swine *Chantal Farmer, Sherbrooke Research Center, Sherbrooke, Quebec, Canada*
1:45	Supplemental Yeast Fermentation Products Effect on Sow Lactation Performance and Post-Partum Recovery Based on Uterine Fluids and Blood Parameters *Brian Richert, Purdue University*
2:20	Break
2:50	The Impact of Nutrition on Barrier Function in the Young Pig *Sheila Jacobi, The Ohio State University*
3:25	The Effect of Medium Chain Fatty Acids on Porcine Reproductive and Respiratory Syndrome Virus *Scott Radcliffe, Purdue University*
4:00	Closing Remarks

Table of Contents

Tilford R. (Tip) Cline

March 15, 1939 – February 12, 2020



Dr. Tip Cline died at his home in West Lafayette, Indiana, at the age of 80. He is survived by his two children, Michelle Cline and Todd Cline, both of West Lafayette, his former wife, Mary Cline Meyer of Petersburg, Indiana, and numerous cousins in Central Illinois and California. He was preceded in death by his parents, Byron Ray and Margaret Thornborrow Cline, and his brother, John Ray Cline.

Tip was born and raised on a livestock and grain farm in West Central Illinois where he graduated from high school in 1956. He subsequently attended and graduated from the University of Illinois with a BS degree in 1960, a MS degree in 1962, and a PhD degree in 1965. After serving in a post-doctoral position at Purdue University, he was hired as an Assistant Professor in the Department of Animal Science and rose to the rank of Professor in 1977. Following a 40-year career at Purdue University, he retired on August 31, 2005 and received Professor Emeritus status.

Although making a career in both research and teaching, Tip primarily distinguished himself by spending the majority of his effort in teaching undergraduate and graduate students. He received numerous recognitions for his teaching efforts, two of which include the American Society of Animal Science (ASAS) Midwestern Section Outstanding Teacher Award in 1976, and the ASAS Fellow Award in Teaching in 1998.

Tip Cline was one of the founders and organizers of the Midwest Swine Nutrition Conference which began in 2001 and continued for 20 years (until interrupted by COVID in 2020). This conference has continually grown and is presented annually, and includes the swine nutrition faculty from the land grant universities of five states (Illinois, Indiana, Kentucky, Michigan and Ohio) as committee members. Dr. Cline served as chairman of the planning committee the first 8 years of the conference's existence.

The Purdue College of Agriculture has established an endowment to honor Dr. Cline. This endowed fund will be used to support students that show an interest in a career in animal nutrition or that are planning to pursue graduate studies in the Department of Animal Sciences. Those who wish to contribute to the **Tip Cline Memorial Endowment** should contact April Shepherd, Director of Development, at agshepherd@purdueforlife.org or 765-494-7435.

Tip Cline is sorely missed by all of those who knew him. His hard work, common-sense approach to research, excellent teaching abilities, sports enthusiasm, and unique humor will long be remembered by his many former students, colleagues, and friends.

Aspects of Sow Nutrition during Transition and Lactation to Improve their Productivity and Feed Efficiency

Peter Kappel Theil
Aarhus University
Aarhus, Denmark
peter.theil@anis.au.dk

Summary

Feed efficiency, productivity and farm profitability are key factors in sustainable pig production, but a suitable measure of feed efficiency is not available for sow herds. Feed level and feed composition should be composed to match the daily requirements for nutrients, but there is a conflict between the use of only one diet during gestation and lactation periods and the fact that the daily requirements change quite dynamically. A two component strategy seems to be a promising approach.

Introduction

Feed costs account for the majority of expenses in pig production, and feed efficiency is essential for farmers to have a profitable production. In weaned and growing/finishing pigs it is easy, even at a farm level, to survey changes in feed efficiency but this is not really possible for sows. At the farm level, feed efficiency of sows can be evaluated based on the total feed consumed per sow per year, but based on this figure it is not really possible to conclude why a herd is not performing well. For instance, a low feed efficiency could be due to an individual farms production of gilts being fed lactation feed (rather than purchasing replacement gilts) and hence included in this calculation. Another reason for low feed efficiency may be "yoyo" sows that mobilize too much from their body depots during lactation and utilize excess gestation feed to rebuild their body during the subsequent gestation. Other obstacles to derive a reliable feed efficiency include differences in weaning age and whether or not milk supplementation is provided for the suckling piglets. Detailed studies on reproductive phases seems to be the way forward to better understand the concept of feed efficiency of reproductive sows, and two component feeding seems to be a promising approach to ensure high productivity and high feed efficiency of reproductive sows.

How Can We Define Feed Efficiency of Sows?

A simple way of considering the feed efficiency of lactating sows would be to express the milk production per kg of feed consumed. Intuitively, this trait should be maximized, to achieve a high feed efficiency analogous to maximizing the gain-to-feed ratio of pigs. However, looking at the classical study by King and Dunkin (1986), where groups of sows were fed increasing levels of feed (1.5 to 4.8 kg/d), reveals that sows with the lowest feed intake appeared to be three times more feed efficient that sows with a high feed intake, simply because they support a much greater proportion of their milk production from body mobilization (Figure 1). At the lowest feed supply (1.5 kg/d) in the study by King and Dunkin (1986) it can be calculated that 64% of the milk was produced from body reserves, whereas it was only 14% at the highest feed supply (4.8 kg/d), whereby as much as 86% of the milk was produced directly from the feed. So—what is the trick to achieve both a high feed efficiency of reproductive sows and to concomitantly improve their productivity? The trick is to allow the sow to produce as much milk (or fetal growth) per kg of feed when accounting for changes within the sow body. Thus, if sows mobilize body tissue during lactation, it is necessary to quantify this on an energy basis to

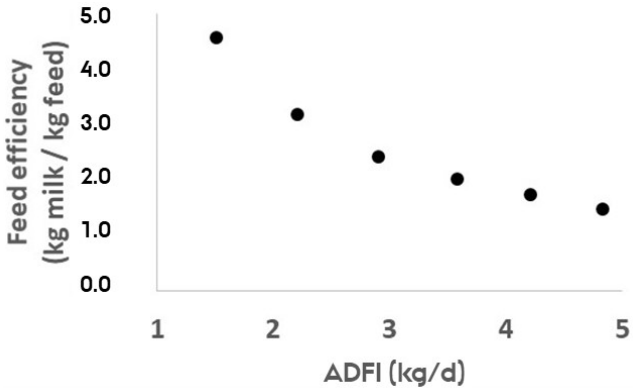


Figure 1. Feed efficiency of lactating sows when ignoring how mobilization from the sow body counteracts improper feeding (modified from King and Dunkin, 1986).

allow correction of e.g. milk being produced from body tissues, as suggested by net energy corrected for body mobilization (NEc) by Pedersen et al. (2019). To feed as optimal as possible, it is necessary to know the live weight of sows, the number of fetuses in gestating sows, and the milk yield of lactating sows. Optimal feeding strategy (feed supply and feed composition) each and every day will give the best feed efficiency, but that is indeed a challenge in production systems when not even the simplest data (live weight) of sows is known.

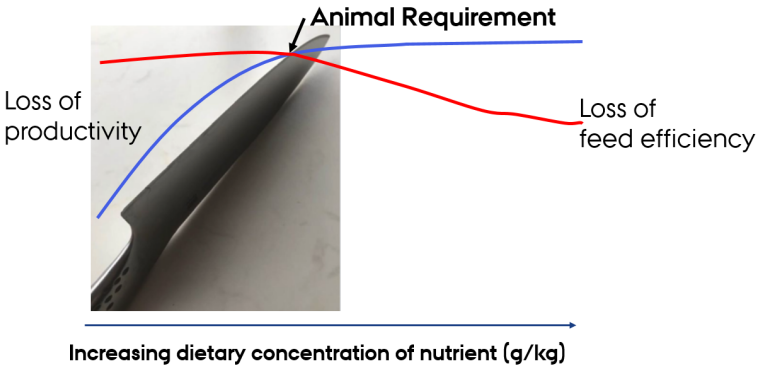


Figure 2. Achieving a high feed efficiency and a high productivity of sows is a challenge because the daily nutrient requirements change dynamically during gestation and lactation periods.

Energy and Lysine Requirements

The requirement of energy and lysine (and many other essential nutrients) changes dynamically during the reproductive cycle (Feyera and Theil 2017; Sola-Oriol and Gasa, 2017). To achieve a high feed efficiency, a high productivity, and a profitable production, it is crucial to feed sows as close as possible to their daily requirement (Figure 2). Phase feeding, or even better multiphase feeding (two component feeding) would be a way forward to maximize sow productivity, feed efficiency and farmer profitability at the same time.

Dietary energy and lysine are the most important dietary factors for pig nutrition and may be seen as the first and second law of pig nutrition. This is also the case for sows. Unfortunately, the daily requirements for energy and lysine do not change proportionally. For instance, the lysine requirement increases day by day due to fetal and mammary growth whereas the energy requirement remains more stable in late gestation (Feyera and Theil, 2017; Sola-Oriol and Gasa, 2017). During lactation, requirements of lysine and energy increases dramatically due to the demand for milk production, but the requirements increase disproportionally because the sow body requires substantial amounts of energy but only negligible amounts of lysine for maintenance. These dynamic changes are a challenge because improper feeding results in lower feed efficiency and/or lower productivity, and both aspects are negatively affecting farmer profitability. If sows are being fed diets with too high dietary protein (or insufficient lysine), excess amounts of the protein is catabolized in the liver and utilized as energy, which lowers the feed efficiency as evaluated using net energy corrected for mobilization (Pedersen et al., 2019; Feyera et al., 2020).

Piglet survival is highly important to maximize the number of weaned pigs per sow per year, and the farrowing process (Feyera et al., 2018) and colostrum and milk produced by the mammary glands are crucial aspects on which to focus if sow productivity is to be enhanced (Quesnel et al., 2012; Theil et al. 2012). While energy supply (1st law of nutrition) seems to be the only determining factor for a successful farrowing (Feyera et al., 2018, 2021; Nielsen et al., 2021), colostrum (Feyera et al., 2021) and milk production (Hojgaard et al., 2020) are dependent on a proper balance between lysine and energy along with an adequate energy intake (i.e., both 1st and 2nd law of nutrition). The requirements of both energy and lysine (and many other nutrients) may be ensured fairly close to the individual requirement if sows are provided two different feed components at each meal, namely a basal diet low in energy and nutrients for the sow body and an energy and nutrient dense component formulated to match the demand for milk production (Pedersen et al., 2016; Feyera et al., 2020).

Mobilization Patterns of Sows

From a feed efficiency point of view, it is indeed of great interest to understand how much energy is mobilized from the body, and why the sow is having a certain mobilizing pattern. In other words – is the sow able to mobilize only fat, when energy is needed and only body protein when lysine (and other amino acids) are needed? Our recent research indicates that the mobilization patterns are rather confined and that lactating sows always mobilize both from fat and muscle tissues if either energy or lysine is inadequately supplied via the diet (Feyera et al., 2020). In another recent study with multi-catheterized sows we found that the liver oxidizes substantial amounts of lysine during lactation the first few hours after feed consumption even though lysine supply was inadequate (Hu et al., 2020). These findings emphasize our need to understand the daily requirements of energy and lysine of reproductive sows, and also the nutrient dynamics between meals.

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Thermo-regulation in Neonatal Piglets—Practical Implications for Survival

Mike Ellis, Katherine Vande Pol, Naomi Willard, Alicia Olivo
Department of Animal Sciences
University of Illinois, Urbana-Champaign 61801
Phone: 217-333-6455
mellis7@illinois.edu

Summary

Neonatal piglets are susceptible to hypothermia immediately after birth which predisposes them to mortality. They are small, have limited body surface insulation, and are born wet into a relatively cold environment, resulting in a high potential for heat loss. In addition, they have low body energy reserves and limited capacity to increase heat production. Consequently, body temperature of newborn piglets declines in the early postnatal period, particularly for piglets of low birth weight that are more susceptible to hypothermia than heavier littermates. Increasing the ambient temperature in the facility on the day of farrowing reduces the extent of the decline in piglet body temperature after birth but may negatively affect the sow. Interventions such as drying and/or warming of piglets at birth are effective at reducing the extent and duration of the postnatal decline in body temperature. In addition, these interventions are relatively more effective in piglets of low than higher birth weight. However, the impact of reducing the extent and duration of the decline in body temperature in piglets after birth on pre-weaning mortality has not been clearly established.

Introduction

The period during and immediately after birth is the most critical in the life of any mammal, including the pig. It is obviously a time of transition from life in the very stable environment of the uterus to the challenges of independent survival in the farrowing pen. During this transition, the piglet experiences a dramatic transformation in all aspects of the physical environment, not least of which are the changes in the climatic conditions that the animal is exposed to, particularly the ambient temperature. The temperature in the uterus is relatively constant and is close to the core body temperature of the sow, which generally fluctuates between 38 and 39°C (Littledike et al., 1979). Ambient temperature levels in farrowing rooms are based on the comfort of the sow. Black et al. (1993) suggested that the thermoneutral zone for sows in lactation was between 15 and 20°C. Similarly, Quiniou and Noblet (1999) proposed that the evaporative critical temperature, above which sows must increase evaporative heat loss via increased respiration rate (in the absence of externally applied moisture), was below 22°C. At higher farrowing room temperatures, sows experience heat stress and decreased performance (Muns et al., 2016). Consequently, farrowing room temperatures are generally maintained at around 18°C during lactation; however, it is common practice to increase temperatures on the day of farrowing to around 22°C to provide a higher temperature for the piglets immediately after birth (PIC, 2018). Nevertheless, at birth, piglets experience a dramatic and extensive decrease in environmental temperature of up to 20°C. This represents a major challenge to survival at a stage in the animal's development when it is ill equipped to deal with this thermoregulatory challenge.

Several studies have suggested an unfavorable relationship between the extent of the decrease in rectal temperature early after birth and pre-weaning mortality. Tuchscherer et al. (2000) found that piglets surviving to weaning showed a lower decline in rectal temperature in the first hour after birth than those that died (0.5 vs. 1.5°C, respectively). Similarly, Rothe (2011) found that piglets that died prior to weaning had lower rectal temperatures (by 1.7°C) and were also lighter (by 0.27 kg) at birth than those that survived. However, these reports were from retrospective analyses of data sets and do not necessarily reflect any causal relationship.

This paper will present a brief general overview of thermoregulation in the pig, specifically focused on the unique aspects related to the neonatal piglet. In addition, recent research aimed at minimizing the effects of temperature challenges on piglets will be reviewed.

Thermal Characteristics of Neonatal Piglets

A comprehensive and excellent review of this subject has been presented by Curtis (1970). In common with all mammals, the pig is a homeotherm, maintaining a relatively constant body temperature in environments in which ambient temperatures can vary widely. However, in comparison to the newborn of other farm livestock species, the neonatal pig is particularly susceptible to hypothermia. They are relatively small at birth and, consequently, have a high body surface area to body mass ratio and, therefore, a relatively high potential for heat loss. They are born into a cool environment and have limited body surface insulation (minimal subcutaneous fat levels and pelage) to control heat transfer from the core to the surface of the body. Furthermore, piglets are born wet, and evaporation of the amniotic fluids requires relatively high amounts of energy and removes a substantial amount of heat from the animal. Collectively, these factors result in newborn piglets having the potential for high levels of heat loss.

Under cold conditions, animals rely on increasing heat production to maintain body temperature; however, neonatal piglets have limited capacity to increase heat production in response to cold stress. Unlike some other mammalian species, piglets do not appear to have brown adipose tissue depots (Trayhurn et al., 1989) and rely on shivering for thermogenesis under cold conditions. However, it would appear that the capacity to increase heat production is limited at birth and subsequently takes some time to develop (Herpin et al., 2002). As a result, piglets are cold susceptible, with a critical temperature of around 34°C at birth (Herpin et al., 2002). However, the comfort zone of the piglet is relatively narrow (Strombach et al., 1973) and they can also experience heat stress. Pigs do not sweat and, consequently, rely on increasing respiration rate to increase evaporative cooling to maintain body temperature at higher environmental temperatures (Strombach et al., 1973). A common commercial practice is to confine piglets under a heat source in the early period after birth and if the temperature of that area is not well regulated, it is not uncommon to observe high respiration rates in piglets.

In a general sense, the different ways that the newborn piglet loses heat to the environment are well understood; however, there has been limited quantification of the relative importance of these under typical conditions. This relative importance will depend on a number of animal factors and also on the environmental conditions. After the initial evaporation of amniotic fluid, evaporative heat loss is mainly from the respiratory tract and is relatively limited, and unless the piglets are in contact with cold surfaces, conductive heat loss is also minimal (Curtis, 1970). Consequently, the majority of heat loss from piglets occurs via either convection or radiation, with the relative importance of these varying with the temperature of the air and solid surfaces in the farrowing pen, respectively (Mount, 1964; 1967). When these two temperatures are similar, heat loss via convection and radiation are also similar. However, if ambient temperature is lower than surface temperature then convective heat loss will be greater than heat loss from radiation and *vice versa* (Mount, 1967). A number of environmental factors can influence both the absolute amount of heat loss from the piglet as well as the relative importance of the various routes of heat loss. For example, increasing air movement over the piglet would be expected to increase both total and convective heat loss.

Tissue insulation, which is provided mainly by subcutaneous fat, and pilo-erection of hair coat and the air trapped within, also, in theory, play a part in limiting heat loss by impeding heat transfer from the body core to the boundary surface of the body. However, because the piglet is born with limited body fat reserves and sparse hair covering, these mechanisms play little if any part in piglet thermoregulation (Curtis, 1970).

The piglet exerts some control over the rate of heat loss by changing the effective temperature at the body surface (physical thermoregulation) and/or by altering its effective surface area by changes in posture and/or behavior (behavioral thermoregulation). Vasoconstriction occurs under cold conditions, reducing the transfer of heat from the body core to the body surface. Mount (1964) showed that when air and wall temperatures were reduced from 30 to 20°C, the skin temperature of piglets decreased from 37 to 33°C, thereby reducing the temperature gradient between the piglet and the environment from 17 to 13°C. Major behavioral changes exhibited by pigs under cold conditions include postural changes to minimize the area of the body surface in contact with the floor, seeking out more favorable microclimates, and group related behaviors such as huddling. However, postural changes, which have been shown to half the amount of heat lost to the floor (Mount, 1967), appear to be more important than huddling for reducing heat loss prior to weaning (Vasdal et al., 2009). It is common practice to provide a warm "creep" area in the farrowing pen which is normally kept at temperatures close to the critical temperature of the piglet. However, neonatal piglets are more attracted to the sow than to a heat source (Hrupka et al., 1998), and temperatures near to the sow will generally be at a lower temperature than that in the creep area.

To maintain a stable body temperature, animals must balance any heat lost to the environment with heat production. Due to the relatively high potential for heat

loss as a result of the factors discussed above, newborn piglets need to have relatively high rates of heat production. Curtis (1970) estimated that newborn piglets at an environmental temperature of 35°C would need to produce approximately three times as much heat per day as the fetal piglet. Typically, farrowing room temperatures are considerably lower than this. However, the metabolic rate of piglets is relatively low in the first day after birth. Piglets do increase metabolic rate when exposed to cold conditions; however, the response is more limited in the first 24 h than in the second day after birth (Mount, 1959; Curtis et al., 1970). Neonatal piglets do not have brown fat depots that are a central component of thermoregulatory responses under cold conditions of newborn in species such as sheep (Oelkrug et al., 2015). They are dependent mainly on glycogen reserves in muscle and liver tissue for metabolism to increase heat production. The capacity to metabolize fat for energy is limited in the first day after birth (Curtis and Rogler, 1970). Ensuring ingestion of colostrum as soon as possible after birth is important for immunity and, ultimately, for the provision of energy. However, it appears that this energy is not available for metabolism for some time after birth and, therefore, has no effect on the thermal-stability of piglets in the early postnatal period (Mount, 1969). In support of this, Le Dividich and Noblet (1981) found that, for piglets kept at a temperature of 18 to 20°C, colostrum intake explained only 3% of the variation in rectal temperature at 8 h after birth but around 45% of the variation in rectal temperature at 15 to 24 h after birth.

Practical Implications for Piglet Survival

Hypothermia immediately after birth is a major predisposing factor for pre-weaning mortality (Panzardi et al., 2009) either directly or from secondary causes such as starvation, crushing, and disease (Devillers et al., 2011). Low body temperature in neonatal piglets is associated with decreased mobility and vigor, a reduced ability to compete with littermates during suckling and, consequently, reduced colostrum consumption (Le Dividich and Noblet, 1981). Therefore, minimizing postnatal temperature decline should improve piglet survival. A program of research has been carried out at the University of Illinois to quantify the extent of the decline in piglet body temperature after birth and identify the major factors influencing this decline under typical commercial production conditions. In addition, practical approaches to minimizing postnatal changes in temperature and the impact of these on pre-weaning mortality have been investigated.

Typical changes in piglet rectal temperature over the first 2 h after birth are illustrated in Figure 1. This is based on the study of Vande Pol et al. (2020a) that evaluated different methods to dry piglets at birth. This study was carried out in a commercial farrowing facility that was representative of industry conditions. The data used in Figure 1 are for untreated piglets on the control treatment. The initial decrease in temperature was substantial, with the minimum temperature, which was at 30 min after birth, being on average 3.7°C below the birth temperature. However, the variation in rectal temperature between individual piglets at each measurement time was also substantial (Figure 1). For example, at birth, the range in temperature between piglets was 2.6°C (from 37.6 to 40.2°C) and by 30 min after birth, this range had increased to 8.8°C (from 30.6 to 39.4°C). These results highlight the major decline in piglet temperature that occurs after birth and the considerable variation between piglets in these changes.

Factors Affecting Postnatal Changes in Piglet Temperature

Birth Weight

A major component of the variation between piglets in postnatal changes in temperature is due to differences in birth weight. This is illustrated in Figure 2, which is also derived from the study of Vande Pol et al. (2020a). For this analysis, the data for untreated piglets was divided into three groups on the basis of birth weight [Light (< 1.0 kg); Medium (1.0 to 1.5 kg); Heavy (> 1.5 kg)]. The maximum weight for the Light group (i.e., 1.0 kg) represents the birth weight below which

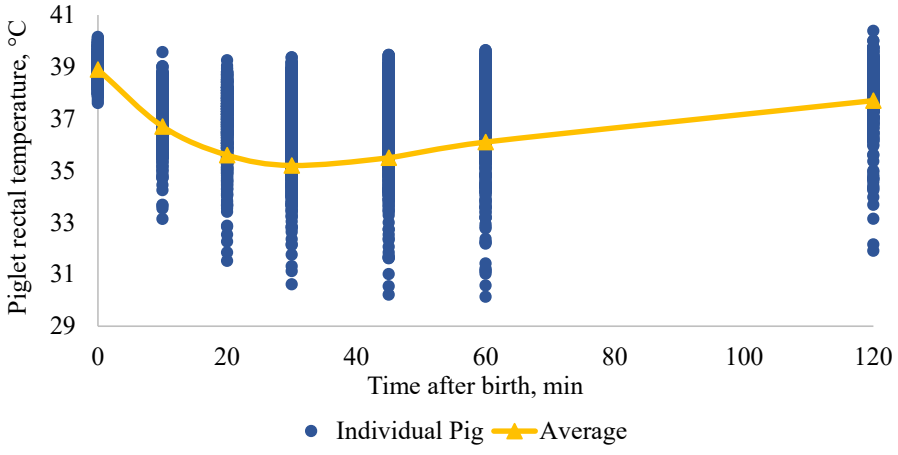

Figure 1. Rectal temperatures of untreated piglets over the first 2 h after birth (Vande Pol et al., 2020a).

piglet mortality increases substantially. The minimum weight for the Heavy group (i.e., 1.5 kg) represented the weight above which piglet mortality is relatively independent of birth weight. The mean birth weight and standard deviation for this population were 1.4 ± 0.37 kg. Rectal temperature declined after birth in all weight groups, however, the extent and duration of the decrease was greater for the Light group (Figure 2). The minimum temperature of the Medium and Heavy groups occurred at 30 min after birth and was 35.1 and 35.7°C, respectively. In contrast, minimum temperature for the Light group occurred at 45 min after birth and was 33.0°C. In addition, the rate of increase in rectal temperature after the minimum was slower in Light than in the Medium and Heavy birth weight groups (Figure 2). This clearly illustrates that piglets with lower birth weight are more susceptible to chilling immediately after birth.

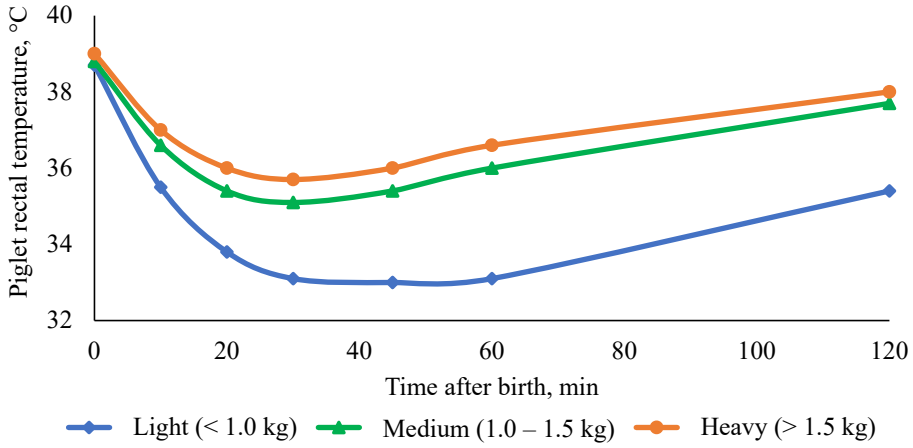

Figure 2. Effect of birth weight on the rectal temperatures of untreated piglets over the first 2 h after birth (Vande Pol et al., 2020a).

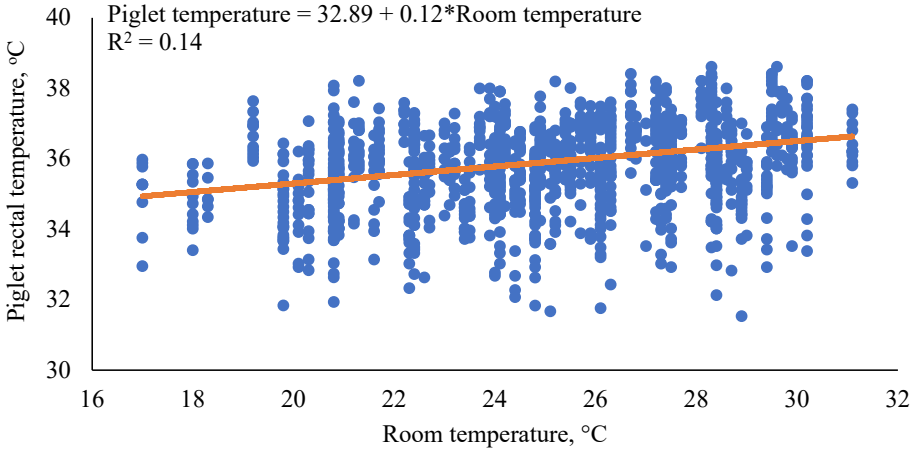

Figure 3. Relationship between farrowing room temperature (at the end of farrowing) and the rectal temperature of untreated piglets at 30 min after birth (Vande Pol et al., 2020a,b; 2021a,b).

Ambient Temperature in the Farrowing Facility

As previously discussed, the larger the temperature gradient between the piglet and the environment, the greater the amount of heat loss from the animal. Conceptually, increasing the farrowing room temperature for the period immediately after birth should reduce heat loss from the piglet, resulting in a less extensive decline in body temperature. However, there has been limited research to prove this concept. We have carried out studies to monitor postnatal changes in piglet rectal temperature in the same commercial farrowing facility in every month of the year; across this time period a wide range of farrowing room temperatures were experienced. This allows for an indirect assessment of the relationship between farrowing room temperature and the extent of the decline in piglet temperature after birth. This relationship for piglet rectal temperature at 30 min after birth (i.e., the time of minimum temperature) is illustrated in Figure 3. The data used was for untreated piglets on the control treatment of four separate studies and is based on a total of 1231 piglets (Vande Pol et al., 2020a,b; 2021a,b). Minimum and maximum farrowing room temperatures in the dataset were 17 and 31°C, respectively, spanning the range typically experienced over the year in the Midwest of the US. Although this relationship was weak (R^2 = 0.14), piglet rectal temperature at 30 min after birth did increase linearly with room temperature (from approximately 35°C at the lowest room temperature to approximately 36.6°C at the highest). Although this is not proof of a causal relationship between room temperature and the extent of the decline in piglet rectal temperature, it does suggest that increasing farrowing room temperature could be beneficial for piglets. However, higher farrowing room temperatures could negatively affect the sows during farrowing (Muns et al., 2016). Therefore, further research is needed to determine the effects of room temperature on both sow and piglet performance.

Drying and Warming of Piglets

Drying and warming piglets at birth are practical approaches that have been used in commercial production to reduce the extent and duration of temperature decline after birth. Drying piglets at birth using materials such as desiccants removes the amniotic fluid from the body surface thereby minimizing evaporative heat loss. Placing the piglets in a warm area reduces the temperature gradient between the piglet and its environment, thereby reducing heat loss from convection and radiation. Because drying and warming have different mechanisms for reducing postnatal temperature decline, in theory, the effects should be additive. The results of a study to evaluate this question are presented in Figure 4 (Vande Pol et al., 2020b). There were four treatments that were applied at birth: Control (piglets not treated); Dried (piglets dried with a cellulose based desiccant); Warmed (piglets placed in a warming box at 35°C for 30 min); and Dried+Warmed. Both the Dried and Warmed treatments substantially reduced the rate and extent of piglet temperature decline in the early postnatal period (Figure 4). For example, piglet rectal temperature at 30 min after birth was 2.0°C greater for these two treatments than for the Control (Figure 4). However, the combination of drying and warming resulted in greater temperatures than the Control treatment (by 2.9°C at 30 min after birth) and also greater temperatures than for either intervention treatment applied independently (Figure 4).

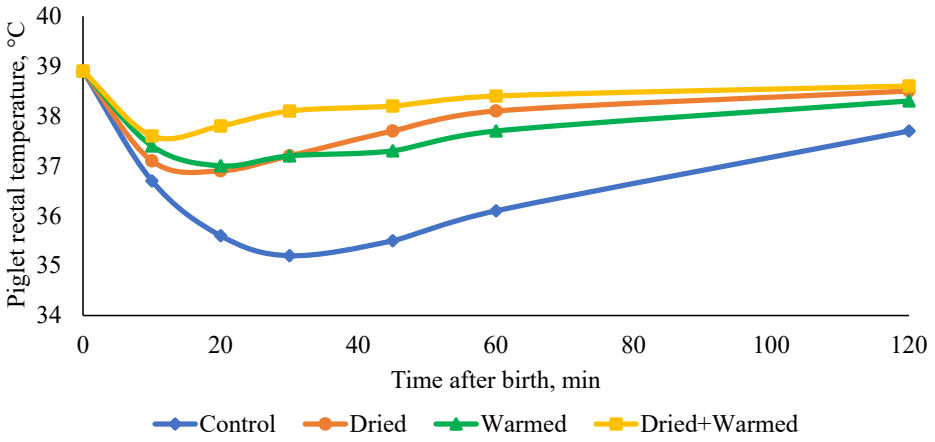

Figure 4. Effect of drying and/or warming piglets at birth on rectal temperatures over the first 2 h after birth (Vande Pol et al., 2020b). Piglets were dried with a cellulose-based desiccant and/or warmed in a warming box for 30 min after birth.

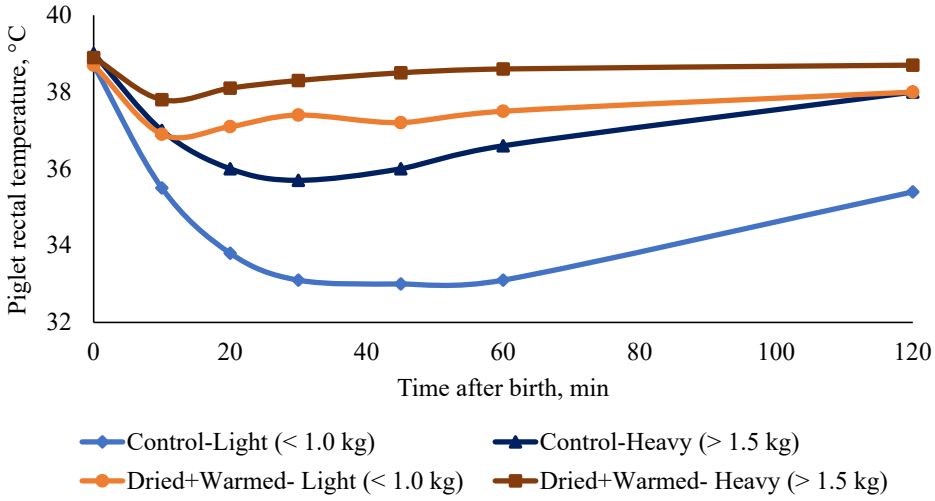

Figure 5. Effect of drying and warming piglets at birth on rectal temperatures over the first 2 h after birth in piglets of Light and Heavy birth weight (Vande Pol et al., 2020b). Piglets were dried with a cellulose-based desiccant and warmed in a warming box for 30 min after birth.

There is also evidence that drying and warming of piglets at birth is relatively more effective at reducing the extent and duration of postnatal temperature decline in low birth weight piglets than in heavier littermates. This is illustrated in Figure 5, which is from the study of Vande Pol et al. (2020b) described above. This figure presents the temperatures between birth and 120 min for Light (< 1.0 kg) and Heavy (> 1.5 kg) birth weight piglets on the Control and Dried+Warmed treatments. The combination of drying and warming resulted in higher rectal temperature than for untreated piglets for both weight groups, however, the increase was greater in Light than Heavy piglets (Figure 5). For example, at 30 min after birth, the rectal temperature of Heavy piglets on the Dried+Warmed treatment was 2.7°C greater than for those on the Control treatment; however, for Light piglets this difference was 4.3°C (Figure 5).

As well as moderating postnatal temperature decline in piglets, interventions such as drying piglets at birth have also been shown to influence aspects of piglet behavior that could be associated with pre-weaning survival. For example, Olivo (2021) showed that piglets that had been dried at birth took less time to achieve

the first suckling compared to untreated piglets. However, in that study, serum immunocrit values measured at 24 h after birth (an index of colostrum consumption) were similar for dried and untreated piglets. In addition, Vande Pol et al. (2020b) found that piglets which were dried and warmed at birth had lower serum immunocrit levels at 24 h after birth than untreated piglets. These results suggest that moderation of the temperature decline in neonatal piglets resulting from drying is not likely to improve the immune status of the piglet.

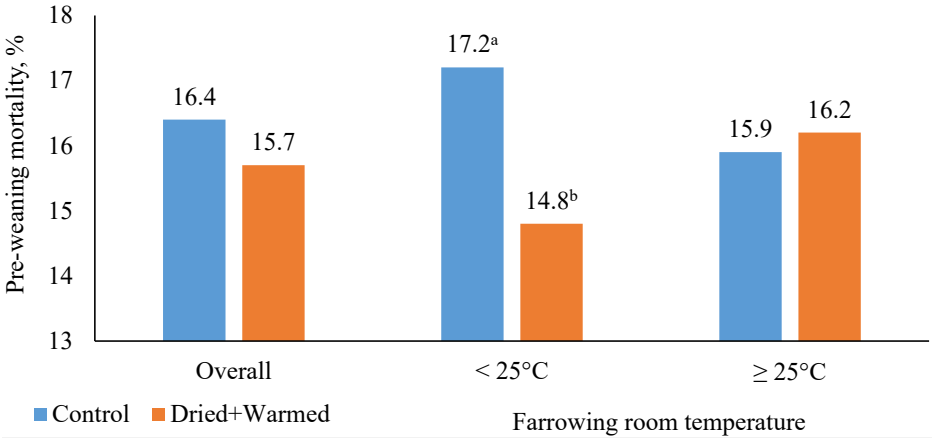

Figure 6. Effect of drying and warming of piglets at birth and of farrowing room temperature on pre-weaning mortality (Vande Pol et al., 2021b). Piglets were dried with a cellulose-based desiccant and warmed in a warming box for 30 min after birth.

Pre-weaning Mortality

Ultimately, the major criterion by which to judge the utility of interventions aimed at reducing the decline in piglet temperature after birth is whether they impact pre-weaning mortality. Unfortunately, research in this area is extremely limited. Vande Pol et al. (2021b) carried out a large-scale study on typical commercial facilities to investigate if the combination of drying and warming of piglets at birth impacted pre-weaning mortality, and the results of this study are presented in Figure 6. While there was no effect of drying and warming on overall pre-weaning mortality, there was an interaction between intervention treatments and farrowing room temperature. When farrowing room temperatures were below 25°C, drying and warming reduced pre-weaning mortality by 2.4 percentage units compared to the untreated control. However, when farrowing room temperatures were above 25°C (temperatures that are experienced during warmer periods of the year), there were no differences between treatments. This suggests that drying and warming may only reduce pre-weaning mortality under cooler farrowing room temperatures, a finding that warrants further investigation.

Causes of pre-weaning mortality are complex and multifactorial and hypothermia immediately after birth is only one potential component. It is remarkable that the majority of piglets are able to recover from relatively extreme temperature declines that would be debilitating in older animals. Further research is needed to more fully understand the impact of moderating the temperature decline in neonatal piglets on subsequent behavior and survival.

Acknowledgements

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The Effect of Different Fat Supplements and Multiple Levels of Vitamin E on Performance and Meat Quality of Pigs Taken to 150 Kg Market Weight

Young Dal Jang†, Ding Wang*, and Merlin D. Lindemann*#
*University of Kentucky, Lexington, KY 40546
†University of Wisconsin River Falls, River Falls, WI, 54022
#Phone: 859-257-7524
#merlin.lindemann@uky.edu

Summary

Due to high usage of DDGS in swine diets, soft pork fat has been an issue in the swine industry. Using different fat sources will modify fatty acid composition in pork and may reduce soft fat issue. As a fat soluble vitamin, vitamin E is a strong antioxidant that reduces oxidative stress in the body, and its absorption and deposition depend on the properties of dietary fat and its fatty acid composition. A study was conducted with growing-finishing pigs fed diets with different fat sources (5% of tallow, corn oil, or coconut oil) and levels of vitamin E supplementation (11 vs. 200 ppm) to a heavy slaughter weight of 150 kg. In the study, increasing vitamin E supplementation level from 11 to 200 ppm increased feed efficiency slightly in the overall period, and increased vitamin E concentrations in plasma, liver, and loin muscle but had minimal effects on fatty acid composition in backfat and antioxidant capacity. Feed efficiency increased with the addition of fat but fatty acid composition in backfat changed with different fat sources depending on the fatty acid composition and intake in which coconut oil increased total saturated fatty acids, corn oil increased total polyunsaturated fatty acids and iodine value, and tallow increased total monounsaturated fatty acids. There was an interaction between vitamin E and fat source in which plasma vitamin E concentrations increased with high level of vitamin E supplementation over time faster in the pigs fed the coconut oil and tallow diets compared to the corn oil diet. These results indicate that increasing dietary vitamin E level is beneficial in enhancing vitamin E status in the body and potentially effective in improving feed efficiency. The addition of fat could improve feed efficiency and modify fatty acid profile in the adipose tissue with a synergistic interaction with vitamin E where tallow and coconut oil containing more saturated and monounsaturated fatty acids may enhance vitamin E absorption in pigs.

Introduction

As global pork consumption has continually increased in the past decades, the increased demand for pork has been met by an increase in both the number of pigs produced and their slaughter weight (**SLW**) and increasing market weight may continue to be a large part of satisfying future pork demand. Meanwhile, the cost pressure from the increasing price of ingredients has driven producers to explore more by-products, among which is the increasing use of distillers dried grains with solubles (**DDGS**), as high as 45% in the swine diets (Cromwell et al., 2011; McClelland et al., 2012). The high content of n-6 unsaturated fatty acids (**UFA**) present in DDGS increases deposition of UFA in tissues resulting in soft-fat pork (Cromwell et al., 2011) and the health issues of pigs (Lauridsen et al., 1999). It has been reported that using various dietary fat sources such as vegetable oils, tallow (**TW**), corn oil, and coconut oil (**CN**) could modify fatty acid (**FA**) composition in tissues and may impact growth performance, carcass traits, and pork quality (Mitchaothai et al., 2007; Chen et al., 2021; Świątkiewicz et al., 2021).

Vitamin E (**VE**), as a strong antioxidant, can be used to reduce oxidative stress in both the live body and final pork products (Lauridsen, 2010; Lauridsen et al., 2013; Ng et al., 2016). Because VE is a fat-soluble vitamin, its absorption and bioavailability depend on the properties of dietary fat (Hoving -Torres et al., 1978). This fact was demonstrated by Prévéraud et al. (2014) in which α-tocopherol concentrations in plasma and tissues of pigs were influenced by dietary fat sources wherein

α-tocopherol concentrations in plasma and tissues actually decreased when pigs were fed high polyunsaturated fatty acid (**PUFA**) diets. However, limited studies have investigated the interaction between VE and fat source when the pigs were fed to a heavy SLW. Therefore, the objective of this project was to identify the impacts of different dietary fat sources and VE supplementation fed to growing-finising pigs grown to a heavy SLW on growth performance, tissue FA profile, plasma and tissue VE concentrations, and antioxidant capacity.

Experimental Procedures

A total of 64 individually-fed pigs (32 barrows, 32 gilts; 28.41 ± 0.83 kg) were blocked by sex and body weight, and then randomly assigned to 1 of 8 dietary treatments in a 4 × 2 factorial arrangement of four fat treatments (including corn starch [CS], TW, distiller's corn oil [DCO] and CN) and two vitamin E (VE) supplementation levels (11 ppm and 200 ppm) in the form of DL (all-rac)-α-tocopheryl acetate (ATA, ROVIMIX E 50 ADS, DSM Nutritional Products, Inc., GA US). All pigs were housed in individual pens with free access to water and feed.

The diets were corn-soybean meal based in mash form and fed to pigs for five weight phases including 25-50 kg (Phase 1), 50-75 kg (Phase 2), 75-100 kg (Phase 3), 100-125 kg (Phase 4), and 125-150 kg (Phase 5), respectively. All experimental diets were formulated to meet or exceed nutrient requirement estimates of NRC (2012) for grow-finishing pigs. Lysine levels for Phase 4 and Phase 5 were calculated with the formula provided by NRC (2012). The fat inclusion level (5%) was based on the amount of corn oil that might be realized from an aggressive use of DDGS (45%) in the finishing diet. The CS diet was formulated to equalize presumed daily intake of non-fat dietary ingredients to the 5% fat-added diets by maintaining constant lysine:ME ratio between the CS and the fat-added diets.

Body weight and feed disappearance were recorded every two weeks to 108 kg and then weekly thereafter to calculate growth performance (ADG, ADFI, and gain to feed ratio [G/F]). Blood samples from each pig were collected initially and at the end of each phase by vena cava puncture into heparinized vacutainer tubes. Plasma samples were obtained by centrifugation at 2500 × g for 20 minutes at 4°C, and stored at –80°C until VE and antioxidant capacity analyses.

Pigs were slaughtered at about 150 kg live weight under USDA inspection. During the process of slaughter, liver subsamples (only left lateral lobe) were collected within 20 minutes after evisceration, flash frozen with liquid nitrogen, and then stored at -80°C for VE and antioxidant capacity analyses.

Once the primal cuts were removed after a 24-h cooler chill, backfat and loin samples from the 10th rib area were collected. The fat samples were vacuum packaged, and then stored at -80°C until FA profile analysis. Two 2.54-cm chops of loin sample were vacuum packaged and stored at -22°C until VE analysis. The FA profiles of backfat were determined by gas chromatography (**GC**), using a Shimadzu gas chromatograph (Model 14 A, Columbia, MD) with a flame ionization detector by a modified procedure from Park and Goins (1994) at the University of Georgia. The iodine value (IV) of backfat was calculated using the equation from Meadus et al. (2010).

The concentrations of VE in plasma and tissue samples was determined by HPLC at the Iowa State University Veterinary Diagnostic Laboratory. The plasma samples were analyzed for superoxide dismutase (**SOD**) and the liver samples were analyzed for SOD, malondialdehyde (**MDA**), glutathione (**GSH**) and glutathione disulfide (**GSSG**) using commercial assay kits purchased from Cayman.

Prior to statistical analyses, all data were evaluated to identify any potential statistical outliers according to the test published by Barnett and Lewis (1974). Data analysis were performed in SAS (SAS Inst. Inc., Gary, NC) by least squares analysis of variance using the generalized linear model (**GLM**) as a randomized complete block design. The individual pig served as the experimental unit. The models used included VE, fat source, sex, block within sex and all possible interactions among VE, fat source, and sex. When interactions between main effects were significant, further least squares mean separations were accomplished using the PDIFF option of SAS to analyze the treatment effects. In addition, plasma VE and SOD concentration data were also analyzed as repeated measures to determine the response trends over time. Statistical differences were established at $P \leq 0.05$, tendencies were established at $P \leq 0.10$. Main effect means are presented in the result tables. When it was present, the effect of sex ($P \leq 0.05$) is superscripted for each measurement in the data table, interactions between sex and treatment effects are also superscripted. To focus attention on statistically relevant data, any *P*-value greater than 0.10 was replaced with "-".

Results

Growth Performance

For the cumulative growth performance (Table 1), no interactions between fat source and VE supplementation were detected ($P > 0.05$). Increasing VE supplementation from 11 to 200 ppm tended ($P = 0.10$) to increase overall G/F. The pigs fed the DCO diet had

Table 1. Effect of different fat source and VE supplementation on cumulative growth performance1 of pigs from 28 to 150 kg

	VE, ppm			Fat source					*P*-value	
Items	**11**	**200**	**SEM**	**CS**	**TW**	**DCO**	**CN**	**SEM**	**VE**	**Fat**
Average daily gain, kg/d										
Phase 1	1.07	1.08	0.020	1.04	1.07	1.10	1.07	0.027	-	-
Phase 1-2	1.07	1.06	0.020	1.04	1.06	1.09	1.08	0.022	-	-
Phase 1-3^{S}	1.05	1.07	0.020	1.04	1.07	1.08	1.05	0.023	-	-
Phase 1-4	1.03	1.06	0.020	1.01^{b}	1.04^{b}	1.11^{a}	1.02^{b}	0.026	-	0.04
Phase 1-5	0.98	1.01	0.020	0.95^{b}	0.98^{b}	1.07^{a}	0.98^{b}	0.026	-	0.02
Average feed intake, kg/d										
Phase 1	2.14	2.12	0.030	2.15	2.07	2.14	2.15	0.040	-	-
Phase 1-2^{S}	2.33	2.33	0.030	2.38	2.26	2.33	2.34	0.041	-	-
Phase 1-3^{S}	2.51	2.53	0.030	2.62^{c}	2.45^{d}	2.52^{cd}	2.48^{d}	0.046	-	0.08
Phase 1-4^{S}	2.62	2.62	0.030	2.72^{a}	2.53^{b}	2.68^{a}	2.54^{b}	0.048	-	0.02
Phase 1-5^{S}	2.67	2.70	0.040	2.79^{a}	2.59^{b}	2.75^{a}	2.59^{b}	0.056	-	0.03
Gain/Feed										
Phase 1	0.502	0.507	0.008	0.487	0.520	0.515	0.498	0.012	-	-
Phase 1-2	0.461	0.456	0.005	0.436^{b}	0.470^{a}	0.468^{a}	0.462^{a}	0.008	-	0.01
Phase 1-3	0.421	0.423	0.005	0.398^{b}	0.435^{a}	0.432^{a}	0.422^{a}	0.007	-	< 0.01
Phase 1-4^{S}	0.395	0.404	0.005	0.372^{b}	0.409^{a}	0.416^{a}	0.401^{a}	0.007	-	< 0.01
Phase 1-5^{S}	0.367	0.378	0.004	0.341^{b}	0.378^{a}	0.390^{a}	0.380^{a}	0.006	0.10	< 0.01

^{a,b}Means within the same row of the fat source effect without a common superscript differ (P < 0.05).
^{c,d}Means within the same row of the fat source effect without a common superscript differ (P < 0.10).
1 CS, corn starch; TW, 5% tallow; DCO, 5% distiller's corn oil; CN, 5% coconut oil. Main effect means are the average of 16 pigs per fat source and 32 pigs per VE level; ^{S} significant sex effect, P < 0.05. No interaction between fat source and VE supplementation was observed. *P*-values greater than 0.10 are replaced with "-".

greater ($P < 0.05$) ADG than the pigs fed the CS, TW, and CN diets in Phase 1-4 and 1-5. The pigs fed the CS diet tended to have greater ADFI than those fed the TW and CN diets in Phase 1-3 ($P = 0.08$) while the pigs fed the CS and DCO diets had greater ADFI than those fed the TW and CN diets in Phase 1-4 and the overall period ($P < 0.05$). The pigs fed the fat-added diets had greater ($P < 0.05$) G/F compared to the pigs fed the CS diet in all phases except for Phase 1.

Tissue Fatty Acid Profile in the Backfat

No interactions between dietary VE supplementation and fat source were observed on FA profile in backfat at slaughter (Table 2) except that increasing VE supplementation level increased C20:4 ($P < 0.05$) when the pigs were fed the DCO diet but not any other diets.

Increasing dietary VE level from 11 to 200 ppm increased C16:1 ($P < 0.05$) and CLA ($P = 0.08$) but decreased C18:0 and C20:0 ($P < 0.05$). Regarding the effect of fat source, the pigs fed the CN diet had the most SFA including C12:0, C14:0, and C16:0 ($P < 0.05$); the pigs fed the DCO diet had most PUFA including C18:2n6, C18:3n3, C20:2, and C20:4 with the greatest IV ($P < 0.05$); and the pigs fed the TW diet had most MUFA including C18:1, C17:0, C18:3n6, and CLA ($P < 0.05$) in the backfat. However, the pigs fed the CN diet had the most C16:1 ($P < 0.05$) whereas the pigs fed the CS diet had the most C18:0 ($P < 0.05$) among different fat treatments.

Plasma and Tissue VE Concentrations

Increasing dietary VE level from 11 to 200 ppm increased VE concentrations in plasma for all phases, liver and loin muscle ($P < 0.05$; Table 3). Regarding the effect of dietary fat source, the pigs fed the CS diet had lower plasma VE concentrations than the pigs fed fat-added diets in Phase 2 and 5 ($P < 0.05$). The pigs fed the CN diet had greater plasma VE concentrations than the pigs fed the CS and TW diets in Phase 3, and the CS, TW and DCO diets in Phase 4. No effect of dietary fat source was observed on VE concentration in liver and loin muscle at slaughter.

With interactions between fat source and VE level ($P < 0.05$; Table 4), increasing VE supplementation level from 11 to 200 ppm increased plasma VE concentrations in which plasma VE concentrations were greater ($P < 0.05$) in the pigs fed the TW and CN diets than the CS and DCO diets in Phase 2 and 5 when 200 ppm VE was supplemented.

Antioxidant Status

With an interaction between fat source and VE supplementation at the end of Phase 2 in plasma SOD activity (Table 4), the pigs fed the CN diet had the lowest ($P < 0.05$) SOD activity among different dietary fat treatments when 200 ppm VE was supplemented, while no effect was detected when dietary VE was 11 ppm. Otherwise, no effect of fat sources and VE was detected on the SOD activity in the plasma (Table 5). No main

Table 2. Effect of different fat source and VE supplementation on fatty acid profile (%) in the backfat[1]

	VE, ppm			Fat source					*P*-value	
Items	**11**	**200**	**SEM**	**CS**	**TW**	**DCO**	**CN**	**SEM**	**VE**	**Fat**
C10:0	0.07	0.07	0.002	0.06^{b}	0.05^{c}	0.05^{a}	0.11^{c}	0.003	-	< 0.01
C12:0	0.45	0.46	0.026	0.07^{b}	0.05^{b}	0.06^{b}	1.62^{a}	0.036	-	< 0.01
C14:0	2.37	2.47	0.047	1.35^{b}	1.39^{b}	1.12^{c}	5.82^{a}	0.066	-	< 0.01
C16:0	25.18	25.18	0.176	25.90^{b}	23.99^{c}	22.08^{d}	28.74^{a}	0.243	-	< 0.01
C16:1	2.37	2.53	0.054	2.20^{c}	2.60^{b}	1.53^{d}	3.48^{a}	0.074	0.03	< 0.01
C17:0	0.28	0.28	0.008	0.23^{b}	0.52^{a}	0.20^{bc}	0.18^{c}	0.012	-	< 0.01
C18:0	14.82	14.16	0.226	16.42^{a}	14.05^{c}	12.49^{d}	15.00^{b}	0.313	0.04	< 0.01
C18:1	40.15	40.36	0.305	43.23^{b}	46.30^{a}	36.02^{c}	35.48^{c}	0.428	-	< 0.01
C18:2n6	11.87	12.09	0.211	8.17^{b}	8.39^{b}	23.40^{a}	7.95^{b}	0.296	-	< 0.01^{G}
C18:3n6	0.08	0.08	0.003	0.07^{b}	0.16^{a}	0.05^{c}	0.04^{c}	0.004	-	< 0.01
C18:3n3	0.44	0.44	0.009	0.36^{b}	0.38^{b}	0.69^{a}	0.33^{c}	0.013	-	< 0.01
CLA	0.16	0.17	0.004	0.09^{b}	0.40^{a}	0.09^{b}	0.08^{b}	0.006	0.08	< 0.01
C20:0^{S}	0.28	0.26	0.005	0.30^{a}	0.26^{bc}	0.28^{ab}	0.24^{c}	0.008	< 0.01	< 0.01^{G}
C20:1	0.83	0.81	0.029	0.98^{a}	0.96^{a}	0.75^{b}	0.60^{c}	0.040	-	< 0.01
C20:2	0.49	0.48	0.011	0.42^{b}	0.33^{c}	0.97^{a}	0.23^{d}	0.016	-	< 0.01^{G}
C20:4^{IS}	0.15	0.15	0.004	0.14^{b}	0.13^{bc}	0.21^{a}	0.12^{c}	0.005	-	< 0.01
∑SFA	43.45	42.87	0.352	44.33^{b}	40.33^{c}	36.29^{d}	51.70^{a}	0.471	-	< 0.01
∑MUFA	43.35	43.71	0.328	46.40^{b}	49.87^{a}	38.30^{c}	39.56^{c}	0.461	-	< 0.01
∑PUFA	13.19	13.42	0.226	9.26^{bc}	9.80^{b}	25.41^{a}	8.75^{c}	0.303	-	< 0.01^{G}
Iodine value	60.89	61.61	0.435	56.61^{c}	60.66^{b}	77.83^{a}	49.90^{d}	0.610	-	< 0.01^{G}

a-d Means within the same row of the fat source effect without a common superscript differ (P < 0.05).
1 CS, corn starch; TW, 5% tallow; DCO, 5% distiller's corn oil; CN, 5% coconut oil. Main effect means are the average of 16 pigs per fat source and 32 pigs per VE level; I significant interaction between fat and VE, P < 0.05; S significant sex effect, P < 0.05; G significant interaction with sex, P < 0.05. *P*-value greater than 0.10 was replaced with "-".

effects and their interaction between dietary fat source and VE supplementation were observed on liver antioxidant status except liver SOD activity wherein the pigs from the DCO treatment had greater liver SOD activity ($P < 0.05$) than the CN treatment.

Discussion

The effect of fat supplementation always involves both energy density and ratio of protein/lysine:energy (Gu and Li, 2003). When protein:energy ratio remained constant with the increasing fat supplementation, the ADG was not affected by fat supplementation either from different sources or levels, while reduced ADFI and increased G/F were consistently reported (Pettigrew et al., 1991; Liu et al., 2018). The general decrease in cumulative feed intake and increase in feed efficiency in the finishing periods with 5% fat addition in the current study are in agreement with Liu et al. (2018) who reported that when the pigs were fed diets with 6% of soybean oil, choice white grease, palm oil, animal-vegetable blend, or TW, there were an improvement in feed efficiency and a decrease in ADFI in most fat sources with no effect in growth rate. However, even though the ratio of SID lysine content and ME density was maintained consistent across all treatments in the current study, 5% fat supplementation increased growth rate during late

Table 3. Main effect of different fat source and VE supplementation on VE concentration1 in plasma and tissue

	VE, ppm			Fat source					*P*-value	
Items	**11**	**200**	**SEM**	**CS**	**TW**	**DCO**	**CN**	**SEM**	**VE**	**Fat**
Plasma, ppm[2]										
Day 0^{S}	2.10	2.25	0.080	2.19	2.19	2.16	2.16	0.111	-	-
Phase 1	1.37	3.89	0.120	2.30^{e}	2.74^{d}	2.89^{d}	2.60^{de}	0.171	< 0.01	0.10
Phase 2^{I}	1.16	3.64	0.130	1.93^{b}	2.60^{a}	2.49^{a}	2.58^{a}	0.186	< 0.01	0.047
Phase 3^{I}	1.97	4.75	0.140	2.54^{c}	3.38^{b}	3.58^{ab}	3.95^{a}	0.192	< 0.01	< 0.01
Phase 4^{I}	1.81	5.20	0.170	2.66^{c}	3.55^{b}	3.48^{b}	4.34^{a}	0.235	< 0.01	< 0.01
Phase 5^{I}	1.96	5.08	0.140	2.89^{b}	3.99^{a}	3.44^{a}	3.75^{a}	0.192	< 0.01	< 0.01
Liver, ppm wet tissue	4.73	21.06	0.670	10.93	13.60	13.41	13.65	0.932	< 0.01	-^{G}
Loin muscle, ppm wet tissue	1.25	2.67	0.080	2.01	2.00	1.87	1.96	0.113	< 0.01	-

a-c Means within the same row of the fat source effect without a common superscript differ (P < 0.05).
d,e Means within the same row of the fat source effect without a common superscript differ (P < 0.10).
1 CS, corn starch; TW, 5% tallow; DCO, 5% distiller's corn oil; CN, 5% coconut oil. Main effect means are the average of 16 pigs per fat source and 32 pigs per VE level; I significant interaction between fat and VE, $P \leq 0.05$; S Significant sex effect, P < 0.05; G significant interaction with sex, P < 0.05. *P*-value greater than 0.10 was replaced with "-".
2 Time effect: linear, P < 0.0001.

Table 4. Individual treatment effect of different fat source and VE supplementation on VE concentration1 in plasma and tissue

VE, ppm:	11					200				*P*-value	
Fat source:	CS	TW	DCO	CN	SEM	CS	TW	DCO	CN	VE	Fat
Plasma[2], ppm											
Day 0	2.20	2.28	2.00	1.93	2.18	2.10	2.33	2.39	0.16	-	-
Phase 1	1.16^{i}	1.29^{i}	1.93^{h}	1.10^{i}	3.44^{g}	4.19^{f}	3.85^{fg}	4.10^{f}	0.24	< 0.01	0.10
Phase 2[I]	0.95^{c}	1.10^{c}	1.66^{c}	0.94^{c}	2.91^{b}	4.16^{a}	3.31^{b}	4.23^{a}	0.26	< 0.01	0.047
Phase 3[I]	1.43^{e}	1.89^{de}	2.70^{d}	1.83^{e}	3.75^{c}	4.94^{b}	4.56^{bc}	6.00^{a}	0.28	< 0.01	< 0.01
Phase 4[I]	1.24^{e}	1.56^{de}	2.34^{d}	2.14^{de}	4.13^{c}	5.46^{b}	4.53^{bc}	6.61^{a}	0.32	< 0.01	< 0.01
Phase 5[I]	1.44^{d}	2.06^{cd}	2.46^{c}	1.79^{cd}	4.35^{b}	5.63^{a}	4.43^{b}	5.71^{a}	0.27	< 0.01	< 0.01
Liver, ppm wet tissue	3.40	4.40	6.31	3.20	18.45	19.90	20.09	23.36	1.38	< 0.01	-[G]
Loin muscle, ppm wet tissue	1.30^{h}	1.39^{h}	1.33^{h}	1.04^{h}	2.71^{fg}	2.73^{fg}	2.43^{g}	2.90^{f}	0.16	< 0.01	-
Plasma SOD, U/ml in Phase 2[I]	5.06^{bc}	4.50^{bc}	4.70^{bc}	5.29^{abc}	6.66^{a}	5.29^{abc}	5.71^{ab}	3.92^{c}	0.53	-	-

[a-e]Means within the same row of the fat source effect without a common superscript differ (P < 0.05).

[1] CS, corn starch; TW, 5% tallow; DCO, 5% distiller's corn oil; CN, 5% coconut oil. Main effect means are the average of 16 pigs per fat source and 32 pigs per VE level; [I] significant interaction between fat and VE, $P \leq 0.05$; [S] significant sex effect, P < 0.05; [G] significant interaction with sex, P < 0.05. *P*-value greater than 0.10 was replaced with "-".

[2] Time effect: linear, P < 0.0001. Interaction between VE and time, P < 0.0001; interaction between fat and time, P < 0.01. Contrast between slopes of plasma VE concentration with time when pigs were fed 200 ppm dietary VE: CN *vs.* CS, P < 0.05; CN *vs.* CO, P < 0.05; TW *vs.* CS, P < 0.05; TW *vs.* CO, P < 0.05.

finishing period with significantly higher growth rate in the DCO treatment than the CS treatment along with increased feed efficiency in all phases except for Phase 1. This result may be somewhat related to the fact that a mean energy value was used for all fats for formulation purposes but actual ME content of DCO is not the same as TW or CN. It is interesting that the DCO supplementation did not reduce feed intake compared with the no-fat diet which actually resulted in significantly increased growth rate in the late finishing periods compared with the other fat-added diets. This effect might be due to an increase in palatability of the DCO diet as was suggested of fat-supplemented diets (Ziggers, 2005) as well as increased energy digestibility with high content of PUFA in the corn oil (Powles et al., 1994).

Increasing dietary VE supplementation level from 11 to 200 ppm tended to increase feed efficiency in overall period. This result agrees with Asghar et al. (1991) and Niculita et al. (2007) that reported a significant increase in growth rate and feed efficiency when the pigs were fed diets with high level of VE (100 to 300 ppm) compared to those fed diets containing 10 to 11 ppm of VE. However, other studies have reported no effect of VE supplementation in pig growth performance (Cannon et al., 1996; Hoving-Bolink et al., 1998; Lauridsen et al.,1999). Although the biological fuctions of VE in pigs are known to be mainly related to tissue integrity and oxidative stress as an antioxidant (Mahan, 2001), the result of the current study indicates that the current VE requirement in NRC (2012) may not be sufficient to maximize growth performance of finishing pigs with a heavy SLW.

In the current research, corresponding to the intake of different individual FAs, the pigs fed the CN diet had the most SFA including C12:0, C14:0, and C16:0; the pigs

Table 5. Effect of different fat source and VE supplementation on antioxidant status1 in pigs

	VE, ppm			Fat source					*P*-value	
Items	11	200	SEM	CS	TW	DCO	CN	SEM	VE	Fat
Plasma SOD[2], U/ml										
Day 0	4.85	4.27	0.290	4.98	4.27	4.34	4.64	0.410	-	-
Phase 1	4.62	4.02	0.280	4.41	4.52	4.19	4.15	0.391	-	-
Phase 2[I]	4.89	5.39	0.270	5.86	4.89	5.20	4.61	0.372	-	-
Phase 3	5.32	5.54	0.230	5.40	5.74	5.39	5.19	0.314	-	-
Phase 4	5.32	5.01	0.280	5.07	5.54	4.99	5.05	0.391	-	-
Phase 5	7.96	7.77	0.350	7.70	7.51	7.95	8.29	0.491	-	-
Liver antioxidants										
GSH, µmol/g protein	80.91	82.82	2.720	81.33	79.74	83.00	83.38	3.765	-	-[G]
GSSG, µmol/g protein	5.93	6.52	0.320	6.73	5.86	6.36	5.96	0.446	-	-[G]
GSH/GSSG	15.02	14.00	0.720	14.03	15.11	14.06	14.83	0.992	-	-
SOD, U/mg protein	23.02	22.24	0.060	22.51^{ab}	22.67^{ab}	24.40^{a}	20.92^{b}	0.794	-	0.03
MDA, nmol/g protein	70.04	71.97	2.850	75.24	70.38	63.87	74.54	3.996	-	-

[a,b]Means within the same row of the fat source effect without a common superscript differ (P < 0.05).

[1] Main effect means are the average of 16 pigs per fat source and 32 pigs per VE level; [I] significant interaction between fat and VE, P = 0.04; [G] significant interaction with sex, P < 0.05. CS, corn starch; TW, 5% tallow; DCO, 5% distiller's corn oil; CN, 5% coconut oil. *P*-value greater than 0.10 was replaced with "-".

[2] Time effect: linear, P < 0.01. No interaction between time and dietary treatments was observed.

fed the DCO diet had most PUFA including C18:2n6 and C18:3n3 with the highest IV; and the pigs fed the TW diet had the most MUFA especially C18:1 in the backfat. However, the content of C16:0 and C16:1 was the highest in the backfat of the pigs fed the CN diet even though the intake of these two FAs was the highest in the pigs fed the TW diet. Besides, the content of C20:2 in the backfat of the pigs fed the DCO diet was the highest although its intake was the highest in pigs fed the CN diet. The increased deposition of PUFA in the adipose tissue with the DCO source was mainly due to the increase in the intake of these FAs or their precurser FAs including C18:2n6 and C18:3n-3 (Ramsay et al., 2001; Tischendorf et al., 2002; Kloareg et al., 2007) resulting in the increased IV. These results indicate that FA composition in adipose tissues are actively modified depending on dietary FA intake, especially for those non-essential FA (Kloareg et al., 2007), thus while the tissue FA profile mostly follows dietary FA profile there are exceptions.

As the most common FA in grains and oilseeds, linoleic acid (C18:2n6), which is relatively low in animal fat but essential for animals, is one of the most widely evaluated FA. In the present study, the C18:2n6 in the backfat of pigs fed the DCO diet was the greatest among fat treatments as a result of the greatest intake of C18:2n6 in the DCO treatment. This result indicated that the deposition of C18:2n6 in the adipose tissues was concentration-dependent and followed the difference in its intake through diets, which agrees with Kloareg et al. (2007). The extent of the change in C18:2n6 content in muscle and adipose tissues has been reported to be the greatest among all the FAs when pigs are fed a diet with vegetable oil compared to animal fat (Mitchaothai et al., 2007; Corino et al., 2008; Browne et al., 2013).

Oleic acid (C18:1) is the most abundant FA in adipose tissues of pigs, its composition in tissues changed with dietary fat sources in a relatively smaller range than C18:2n6 in this study. Although there was also a large difference in C18:1 intake among different fat sources with the highest intake in the TW treatment, which was 3.9 times (or 390%) higher than that in the CS treatment, the C18:1 in backfat of the TW treatment was only 6% higher that that in the CS treatment. Paton and Ntambi (2009) reported that the stearoyl-CoA desaturase (SCD) gene is responsible for catalyzing the conversion of SFA to MUFA and high C18:1 may inhibit its expression whereas high carbohydrate in the diet may stimulate its expression. Therefore, the highest intake of C18:1, one of the MUFA, in the TW treatment may reduce SCD gene expression and high carbohydrate content in the CS treatment may induce its expression resulting in a smaller difference in C18:1 content in backfat between the TW and CS treatments than expected. Additionally, while the intake of C18:1 in the CS treatment was the lowest, the lowest content of C18:1 in the backfat was observed in pigs from the DCO and CN treatments. For the DCO, this result demonstrated that the high intake of PUFA probably inhibited the SCD gene expression resulting in limited conversion of SFA to MUFA (Ntambi, 1999; Paton and Ntambi, 2009). For the CN, Świątkiewicz et al. (2021) reported that although a CN containing diet contained higher SFA but lower MUFA than the control group, the MUFA content in pork was similar between them possibly resulting from the fact that C12:0 and C14:0, which are the highest among all SFA in the CN, were mainly from the diet but C16:0, C18:0 and MUFA were mainly from biosynthesis in the animals.

Another highly abundant FA in adipose tissues of pigs is palmitic acid (C16:0), which changes to an even smaller extent than C18:1 and C18:2n6 (again, because this FA in the body may be mainly from the biosynthesis [Świątkiewicz et al., 2021]), but did change consistently when dietary fat sources changed. In this study, the intake of C16:0 was the highest in pigs fed the TW diet while lowest in pigs fed the CN diet among the three dietary fat sources but the content of C16:0 in the backfat was the highest in the pigs fed the CN diet. The C16:0 can be actively synthesized endogenously via *de novo* lipogenesis, and it is the end product of lipogenesis in the cytoplasm of cells. This result in the current study may be explained by the fact that the more UFA in the diets, the lower the *de novo* synthesis of fat (Chen et al., 2021).

Another major FA comprising adipose tissues is stearic acid (C18:0), which also changes with variations in dietary FA profile, but to a much smaller magnitude than C18:1 and C18:2n6 and a relatively larger extent than C16:0. Different from the pattern of C16:0, the C18:0 was the lowest in the pigs fed the DCO diet where the intake of C18:0 was also the lowest among the three dietary fat sources. A similar result was previously reported when animal fats were compared to vegetable oils, significant differences were reported with lower C18:0 content in adipose tissue of pigs in vegetable oil treatments than animal fat treatments (Apple et al., 2009).

As expected, the VE supplementation displayed very limited effect on the FA profile of adipose tissues only in C16:1, C18:0, C20:0, and CLA in the backfat. The results for CLA and C20:0, while exhibiting at least a statistical tendency, were of minor magnitude.

In the current study, VE concentration in plasma and tissues (liver and loin muscle) increased when dietary VE level increased from 11 to 200 ppm. Difference in plasma VE concentrations increased with the increasing duration of feeding diets containing 200 ppm VE compared with 11 ppm VE. As previous data

have demonstrated, VE deposition increases linearly with increasing dietary VE supplementation (Monahan et al., 1990; Yang et al., 2009; Lauridsen, 2010). Regarding the interaction between VE and fat source in plasma VE concentrations, when the 200 ppm of VE was supplemented to fat-added diets, plasma VE concentrations increased faster and greater in pigs fed the CN and TW diets compared to pigs fed the CS and DCO diets. This result is in agreement with previous studies, where the VE concentrations in plasma and tissues decreased when pigs were fed high PUFA diet compared to no fat-added diet, and increased when pigs were fed high SFA diet (Prévéraud et al., 2014; Prévéraud et al., 2015). The MUFA content, which is high in the TW, was reported to be positively associated with tissue VE concentrations, due to the favorable role of oleic acid (C18:1) in the secretion of VE enriched chylomicrons (Prévéraud et al., 2015). The VE emulsified in medium-chain triglycerides, which is high in the CN with the SFA, has been demonstrated to have better gastrointestinal absorption compared to long-chain triglycerides (Gallo-Torres et al., 1978). On the other hand, due to the higher digestibility of n-6 FA than other classes, the n-6 FA also enhances the deposition of VE in the tissues although most PUFA, especially n-3 PUFA, may reduce VE deposition (Prévéraud et al., 2015).

Although no effect of fat source and the interaction between fat source and VE were observed, the pigs fed the CN diet had numerically more VE in the liver by 26.6% and slightly more in loin muscle than pigs fed the CS diet within the 200 ppm VE treatments, which agrees with the interaction between fat source and VE in plasma.

Regarding antioxidant capacity, an interaction between fat source and VE supplementation was detected in plasma SOD activity at the end of Phase 2, wherein pigs fed the CN diet had the lowest activity among dietary fat treatments when 200 ppm VE was supplemented. In the liver, pigs from the DCO treatment had higher SOD activity than pigs from the CN treatment, irrespective of VE supplementation. The highest content of free FA in the DCO used in the current study reflected the potential oxidation of this fat source whereas the CN had the lowest content of free FA among the fat sources. Upon the intake of oxidized fat, liver antioxidant activity is reported to increase due to greater oxidative stress (Hung et al., 2017). However, the lack of difference in plasma and liver antioxidant capacity between the VE treatments, does not agree with previous studies that reported improved enzymatic antioxidants systems including SOD, catalase, and glutathione peroxidase with increasing VE supplementation level (Lauridsen et al., 1999; Lauridsen, 2010; Cheng et al., 2017). This disparity might be partly due to a well-controlled environment in the current study, which may have maintained a low total stress level.

In conclusion, increasing dietary VE level from 11 to 200 ppm slightly increased feed efficiency in the overall period while the addition of fat clearly increased feed efficiency. Dietary VE supplementation did not affect FA profile in backfat consistently while the changes in most of the FA profile in backfat followed the FA composition of the different fat sources and its intake. The high level of VE supplementation increased plasma, liver and muscle VE concentrations and dietary fat sources affected plasma VE concentrations with an interaction with the VE supplementation level where the TW and CN increased the VE absorption and deposition greater than the DCO.

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The Effect of Feeding High Oleic Soy Oil in Combination with DDGS on Meat Quality

Bailey Harsh and Katelyn Gaffield
Department of Animal Sciences
University of Illinois, Urbana-Champaign 61801
Phone: 217-244-1694
bharsh2@illinois.edu

Summary

*High oleic soybean oil (**HOSO**) is a new soybean variety developed to have improved nutritional and functional properties. Containing a substantially greater proportion of monounsaturated fatty acids (**MUFA**) than commodity soybean oil, HOSO represents a modified fatty acid profile that may appeal to health-conscious consumers while potentially mitigating negative meat quality and bacon processing problems when used in swine diets. Due to its modified fatty acid profile, HOSO is already being integrating into the foodservice industry. Further, high oleic soybeans are predicted to be the fourth-largest grain and oilseed crop in U.S. within the next decade. Recent research in our lab demonstrated including HOSO in growing-finishing pig diets improved growth performance and resulted in fatter, heavier carcasses. Furthermore, including HOSO in swine diets had no negative implications on pork quality. Increases in pork loin marbling and redness may suggest potential improvements in loin appearance. Finally, feeding HOSO to pigs resulted in altered pork fatty acid composition with bellies containing a greater proportion of MUFA and lesser proportion of poly unsaturated fatty acids (**PUFA**) as graded levels of HOSO were added to diets. As a result, pigs fed the HOSO diets produced thicker bellies with greater flop distances compared to pigs fed dried distillers' grains with solubles (**DDGS**). Ultimately, feeding HOSO as a dietary feed ingredient for pigs has the potential to improve specific growth performance, loin quality, and fresh belly characteristics.*

Introduction

Feeding supplemental fat to growing-finishing pigs can result in improved feed efficiency and reduced feed intake (NRC, 2012; Engel et al., 2001), however, dietary fats are also a key determinant in the composition of pork fat (Azain, 2001). Inclusion of unsaturated fats in swine diets, especially PUFA like those present in DDGS, alters carcass fat composition which may reduce fat quality, leading to belly and bacon processing issues (Xu et al., 2010; White et al., 2009; Ellis and McKeith, 1999). This has led to greater interest in the use of MUFA to minimize meat quality problems. Unfortunately, many oils with increased MUFA also contain relatively high PUFA concentrations (Miller et al., 1990).

Unlike commodity soybean and canola oils, HOSO contains approximately 75% oleic acid and only 7% linoleic acid (United Soybean Board, 2021). Therefore, the majority of the oil is MUFA and contains only limited amounts of the PUFA linoleic acid, the fatty acid regarded as having the greatest influence on fat quality (Wood, 1984). Additionally, greater availability of high oleic soybeans is expected given the enhanced functionality of HOSO in food manufacturing and foodservice applications (Qualisoy, 2021). As production of HOSO continues to increase, there will be greater opportunities for use in the swine industry. While incorporating HOSO into ruminant diets did not alter growth performance or carcass characteristics (Belon et al., 2021), no data are available characterizing the use of HOSO in swine diets.

The primary objective of this study was to determine the effect of feeding HOSO to growing-finishing pigs on growth performance, carcass composition and cutting yield, as well as loin and belly quality. Furthermore, the study aimed to determine the optimal inclusion level of HOSO in growing-finishing diets to maximize growth potential while limiting negative effects on carcass performance. Overall, it was hypothesized that feeding HOSO as a dietary substitute for DDGS would not negatively impact growth performance or carcass cutability.

Experimental Procedures

Animals and Experimental Design

A total of 288 pigs were raised in two separate blocks of equal size. For each block, 4 pigs per pen were housed in 36 pens. A total of 9 pens per dietary treatment were represented for each block. The experimental design was a 2 x 4 factorial arrangement of sex and dietary treatments. Pigs were weighed and allocated to treatments by sex and weight to minimize between-pen variation in starting weight. Pigs were fed for 98 d using a 3-phase feeding system. A grower diet was fed to the pens from d 0 to d 35, an early finisher diet from d 36 to d 70, and a late finisher diet from d 71 to d 98 (Tables 1 and 2).

The first block of pigs (PIC 1050 L2 × L3) had initial BW of 35.7 ± 4.51 kg. These pigs were housed in three separate barns containing fully-slatted floors. Each pen was 1.60 m × 3.96 m (1.58 m^2/pig) and contained a double-lid, dry-box feeder fastened to the side gate and one nipple drinker. In each barn, there were 3 replicates of each dietary treatment. In the second block, pigs (PIC 359 sires × PIC Camborough females) had an initial body weight of 25.1 ± 4.59 kg. These pigs were housed in a single barn containing all 36 pens with partially-slatted flooring. Each pen was 1.83 m × 2.59 m (1.18 m^2/pig) and contained a single-space, dry-box feeder and a nipple drinker.

Dietary Treatments and Sample Analyses

Four dietary treatments including a reference diet consisting of 25% DDGS and treatments containing 2% high oleic soybean oil (**HOSO2**), 4% high oleic soybean oil (**HOSO4**), and 6% high oleic soybean oil (**HOSO6**) were used in this study. The reference diet was formulated with 25% DDGS as it was acknowledged that feeding below 30% DDGS will not negatively impact growth rate or feed efficiency (Xu et al., 2010). Furthermore, DDGS contains approximately 8% corn oil which would equate to a 2% corn oil inclusion when included at 25% of the diet (NRC, 2012). This allowed for direct comparisons of DDGS and HOSO at the same oil inclusion to determine the effects of increasing HOSO inclusion. Samples of both HOSO and DDGS ingredients (Table 3) were collected and analyzed.

Experimental Outcomes

Pigs were individually weighed on d 0, 35, 70, and 98 of the trial. Feed consumption was recorded throughout the trial to calculate pen average daily feed intake (**ADFI**) and gain to feed ratio (**G:F**). Day 98 was con-

Table 1. Ingredient and calculated composition of phased treatment diets, as-is basis[1]

	Phase 1				Phase 2				Phase 3			
Ingredient, %	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**
Corn	50.53	72.99	70.99	68.98	54.98	77.45	75.44	73.43	57.53	80.05	78.01	75.98
Soybean meal	22.00	22.00	22.20	22.00	18.00	18.00	18.00	18.00	16.00	16.00	16.00	16.00
DDGS	25.00	0.00	0.00	0.00	25.00	0.00	0.00	0.00	25.00	0.00	0.00	0.00
High oleic soybean oil	0.00	2.00	4.00	6.00	0.00	2.00	4.00	6.00	0.00	2.00	4.00	6.00
Limestone	1.80	0.84	0.84	0.84	0.98	0.75	0.75	0.75	1.00	0.76	0.76	0.75
Dicalcium phosphate	0.64	1.10	1.10	1.10	0.40	0.84	0.84	0.84	0.10	0.50	0.54	0.55
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.05	0.05	0.05	0.05
Trace mineral premix[3]	0.35	0.35	0.35	0.35	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine HCL	0.28	0.39	0.39	0.40	0.22	0.33	0.33	0.34	0.00	0.11	0.11	0.12
DL-Methionine	0.00	0.08	0.08	0.08	0.00	0.08	0.08	0.08	0.00	0.08	0.09	0.09
L-Threonine	0.00	0.13	0.13	0.14	0.00	0.13	0.14	0.14	0.00	0.13	0.13	0.14
Antioxidant[4]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Calculated Composition												
ME, kcal/kg	3,107	3,385	3,488	3,592	3,123	3,400	3,504	3,607	3,141	3,421	3,523	3,626
CP, %	21.09	15.38	15.24	15.09	19.59	13.88	13.74	13.59	18.86	13.16	13.01	12.86
SID Lys, %	0.99	0.99	0.99	0.99	0.85	0.85	0.85	0.85	0.63	0.63	0.63	0.63
Ca, %	0.65	0.65	0.65	0.65	0.54	0.54	0.54	0.54	0.47	0.47	0.47	0.47
P,%	0.56	0.55	0.54	0.54	0.49	0.48	0.48	0.47	0.43	0.41	0.42	0.41

1 Phase 1, 2, and 3 diets were fed from study d 0 to 35, d 36 to 70, and d 70 to 90, respectively.

2 Provided the following per kg of diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg.

3 Provided the following per kilogram of diet: Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

4 Santoquin 6, Novus International, Inc., St. Louis, MO, USA.

Table 2. Analyzed composition of dietary treatments, DM basis[1]

	Phase 1				Phase 2				Phase 3			
Item	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**
DM, %	87.84	87.71	88.05	88.20	86.90	86.99	87.16	87.71	87.03	86.84	87.19	87.29
CP, %	25.61	18.49	18.63	17.95	22.23	16.74	16.66	16.08	21.24	16.08	15.59	15.30
Lys, %	1.56	1.35	1.37	1.29	1.16	1.15	1.14	1.10	0.99	0.95	0.95	0.93
Ash, %	5.95	4.86	5.32	5.56	5.39	4.66	4.59	4.42	4.87	4.33	4.09	3.71
Ca, %	0.58	0.53	0.59	0.61	0.56	0.49	0.56	0.53	0.46	0.46	0.55	0.43
P, %	0.62	0.54	0.56	0.46	0.52	0.39	0.46	0.39	0.44	0.39	0.42	0.39
Fat, %	4.97	6.21	8.60	10.72	4.92	6.18	7.78	10.39	5.25	6.40	8.25	10.26
FA, mg/g[2]												
C8:0, caprylic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C16:0, palmitic	9.81	7.77	9.10	10.45	10.30	8.63	9.55	10.03	10.20	8.73	9.37	10.93
C16:1, palmitoleic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C17:0, margaric	ND	0.20	0.40	0.56	ND	0.22	0.37	0.53	ND	0.23	0.38	0.58
C18:0, stearic	1.59	1.79	2.66	3.39	1.58	1.90	2.62	3.16	1.56	1.88	2.62	3.45
C18:1, oleic	15.59	27.46	48.22	65.64	17.11	30.81	48.09	61.92	16.55	31.19	48.46	67.77
C18:2, linoleic	35.58	25.03	25.65	26.89	37.53	27.61	27.05	25.53	36.27	27.73	25.60	27.49
C18:3, linolenic	3.14	2.96	3.45	4.01	2.98	3.10	3.34	3.73	2.82	2.97	3.19	3.97
C19:0, nonadecanoic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:0, arachidic	0.25	0.23	0.31	0.38	0.25	0.25	0.32	0.38	0.27	0.25	0.31	0.40
C20:1, gadoleic	0.22	0.22	0.41	0.54	0.20	0.22	0.40	0.49	0.19	0.24	0.44	0.52
C22:0, behenic	ND	ND	0.28	0.35	ND	ND	0.27	0.35	ND	ND	0.28	0.37
C24:0, lignoceric	0.20	0.19	0.22	0.26	0.21	0.18	0.23	0.23	0.20	0.19	0.21	0.26
Total SFA[3]	11.86	10.17	12.97	15.39	12.34	11.17	13.37	14.68	12.23	11.26	13.16	15.99
Total MUFA[4]	16.50	28.56	50.00	67.95	18.01	31.99	49.84	64.08	17.42	32.39	50.24	70.08
Total PUFA[5]	38.72	27.99	29.10	30.90	40.51	30.70	30.38	29.26	39.09	30.71	28.78	31.47
IV ACOS[6]	125.24	113.36	104.72	101.07	124.56	112.93	105.14	100.95	123.89	112.49	103.97	100.60

[1] Phase 1, 2, and 3 diets were fed from study d 0 to 35, d 36 to 70, and d 70 to 90, respectively.
[2] FA per gram of diet.
[3] Total SFA = ([C8:0] + [C14:0] + [C15:0] + [C16:0] + [C17:0] + [C18:0] + [C19:0] + [C20:0] + [C21:0] + [C22:0] + [C24:0]); brackets indicate concentration.
[4] Total MUFA = ([C14:1] + [C16:1] + [C18:1trans-9] + [C18:1n-9] + [C18:1n-7] + [C19:1] + [C20:1] + [C21:1]); brackets indicate concentration.
[5] Total PUFA = ([C18:2n-6] + [C18:3n-6] + [C18:3n-3] + [C20:2n-6] + [C20:3n-6] + [C20:3n-3] + [C20:4n-6] + [C20:5n-3] + [C22:5n-3] + [C22:6n-3]); brackets indicate concentration.
[6] Iodine value = C16:1 (0.95) + C18:1 (0.86) + C18:2 (1.732) + C18:3 (2.616) + C20:1 (0.785) + C22:1 (0.723) (AOCS, 1998).

sidered the end of the trial and overall average daily gain (**ADG**), ADFI, and G:F was calculated. On d 98, the heaviest pig from each pen was selected resulting in 36 pigs from each block (72 total) being transported to the University of Illinois Meat Science Laboratory for slaughter on d 99. The second heaviest pig was transported on d 100 which included 36 pigs from each block (72 total) and slaughtered on d 101 at the University of Illinois Meat Science Laboratory.

Pigs were held in lairage for a minimum of 16 h prior to slaughter and provided ad libitum access to water but not feed. Ending live weight (**ELW**) was determined by weighing pigs immediately prior to slaughter. Slaughter occurred under the supervision of the Food Safety and Inspection Service of the United States Department of Agriculture. Pigs were immobilized using head-to-heart electrical stunning and terminated via exsanguination. Approximately 45 min postmortem, carcasses with internal leaf fat were weighed to determine hot carcass weight (**HCW**). Carcass yield was calculated and expressed as a percentage, dividing HCW by ELW. Carcass composition indicators were evaluated on the left side of carcasses, which were chilled at 4°C for a minimum of 20 hours.

Table 3. Composition of dried distillers' grains with solubles (DDGS) and high oleic soybean oil (HOSO)[1]

	DDGS	**HOSO**
Crude fat, %	5.64	99.48
Fatty acids, % of extractable lipid	92.10	81.60
Free fatty acids, %	6.73	0.04
Moisture and volatile matter, %	46.80	0.11
Unsaponifiable matter, %	1.38	0.34
Thiobarbituric acid value[2]	36.92	14.10
Peroxide value, mEq/kg	13.60	0.80
Oil stability index[3], h	4.23	43.26
Insoluble impurities, %	.	0.16
p-Anisidine value, %	7.39	0.62

[1] Analyzed by Barrow-Agee, Memphis, TN (AOCS, 2011).
[2] There are no units for TBA value.
[3] At 100°C of extracted fat.

The method outlined by Boler et al. (2011) was followed for carcass fabrication and determination of bone-in cutting yield, bone-in lean cutting yield, and boneless carcass cutting yield. Carcass cutability was expressed as a percentage of chilled side weight. At 1 d postmortem, loins were re-faced at the longissimus

Table 4. Main effects of diet and sex on daily gain and feed intake[1]

	Dietary treatment					Sex			P-value		
Item	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**	**SEM**	**Barrow**	**Gilt**	**SEM**	**Diet**	**Sex**	**Diet × Sex**
[2]Pens, n	18	18	18	18		36	36				
Phase 1 (d 0-35)											
BW d 0, kg	30.47	30.27	30.28	30.61	5.36	30.99	29.83	5.35	0.79	< 0.001	0.91
ADG, kg/d	0.91	0.87	0.89	0.92	0.13	0.96	0.84	0.13	0.42	< 0.001	0.23
ADFI, kg/d	1.94	1.90	1.87	1.88	0.35	2.02	1.78	0.35	0.71	< 0.001	0.92
G:F	0.468	0.467	0.480	0.492	0.02	0.478	0.476	0.02	0.22	0.80	0.61
BW d 35, kg	62.15	60.87	61.38	62.65	9.85	64.39	59.14	9.83	0.39	< 0.001	0.42
Phase 2 (d 36-70)											
ADG, kg/d	1.10	1.09	1.09	1.09	0.02	1.18	1.00	0.01	0.93	< 0.001	0.63
ADFI, kg/d	3.04[a]	2.79[b]	2.81[b]	2.65[c]	0.20	3.09	2.56	0.20	< 0.001	< 0.001	0.07
G:F	0.364[c]	0.392[b]	0.392[b]	0.413[a]	0.03	0.387	0.394	0.02	< 0.001	0.33	0.34
BW d 70, kg	100.74	98.94	99.60	100.62	10.14	105.77	94.19	10.11	0.63	< 0.001	0.40
Phase 3 (d 71-98)											
ADG, kg/d	1.00	0.97	1.00	1.04	0.05	1.07	0.94	0.04	0.35	< 0.001	0.44
ADFI, kg/d	3.44[a]	3.08[b]	3.18[b]	3.07[b]	0.07	3.47	2.92	0.06	< 0.001	< 0.001	0.37
G:F	0.293[c]	0.320[b]	0.316[bc]	0.346[a]	0.01	0.311	0.326	0.01	< 0.01	0.09	0.37
BW d 98, kg	129.23	126.67	127.91	130.28	9.35	136.14	120.90	9.29	0.33	< 0.001	0.37
Overall (d 0-98)											
ADG, kg/d	1.02	0.96	1.00	1.00	0.05	1.05	0.94	0.04	0.17	< 0.001	0.29
ADFI, kg/d	2.76[a]	2.55[b]	2.58[b]	2.48[b]	0.21	2.81	2.37	0.20	< 0.001	< 0.001	0.37
G:F	0.371[b]	0.379[b]	0.389[ab]	0.409[a]	0.02	0.377	0.398	0.01	0.02	0.02	0.86

[1] Different superscript letters within the same row reflect dietary treatment differences (P ≤ 0.05)
[2] Each pen housed 4 pigs of the same sex.

thoracis surface posterior to the 10th rib and consecutive 2.54 cm chops were hand cut for the evaluation of all subsequent quality traits. Instrumental color, visual color, visual marbling, subjective firmness, ultimate pH, cook loss and Warner-Bratzler shear force were determined using the methods outlined by Lowell et al. (2018). Instrumental color was measured with a Minolta CR-400 Chroma meter (Minolta Camera Co., Ltd., Osaka, Japan) using a D65 light source, 2° observer angle, an 8 mm aperture, and calibrated using a white tile. Visual color and marbling scores (NPPC, 1999), and subjective firmness scores (NPPC, 1991) were collected by the same technician for both blocks of pigs. Trained sensory panels were conducted as described by Richardson et al. (2018). Panelists were selected from personnel from the University of Illinois Meat Science Laboratory trained using the Sensory Guidelines from the American Meat Science Association (AMSA, 2016). Procedures outlined by Kyle et al. (2014) were used in the determination of fresh belly characteristics and adipose tissue sampling. Adipose tissue samples were used to prepare fatty acid methyl esters using the procedure outlined by Lepage and Roy (1986).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. In., Cary, NC) as a 2 × 4 factorial arrangement of treatments. Pen (n = 72) served as the experimental unit for all variables. Fixed effects were diet, sex, and the interaction between diet and sex. Block and barn nested within block served as the random effects. Effect of diet, sex, and the interaction between diet and sex was considered significant at $P < 0.05$. Least squares means were separated using a probability of difference (**PDIFF**) statement in the MIXED procedure of SAS.

Results

Growth Performance

There were no interactions ($P \geq 0.07$) between dietary treatment and sex for any performance characteristics throughout the trial (Table 4). During phase 2, pigs fed the DDGS diet had greater ($P \leq 0.01$) ADFI than all other treatments while both HOSO2 and HOSO4 had greater ($P \leq 0.03$) ADFI compared with HOSO6. A similar pattern was present during phase 3, with pigs fed the DDGS diet had a greater ADFI ($P \leq 0.01$) compared with pigs fed the HOSO diets, while pigs fed the HOSO diets did not differ ($P > 0.05$) from each other. Differences in both phase 2 and phase 3 translated into overall differences throughout the trial. Pigs fed the DDGS diet had a greater ($P \leq 0.01$) overall ADFI than HOSO2, HOSO4 and HOSO6 diets. However, ADFI was not different ($P > 0.05$) among HOSO-fed pigs. Pigs fed the HOSO6 diet had greater ($P \leq 0.03$) overall G:F than pigs fed DDGS and HOSO2 diets but did not differ ($P > 0.05$) from pigs fed HOSO4.

Table 5. Main effects of diet and sex on carcass characteristics of pigs slaughtered under university conditions[1]

	Dietary treatment					Sex			P-value		
Item	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**	**SEM**	**Barrow**	**Gilt**	**SEM**	**Diet**	**Sex**	**Diet x Sex**
Pens, n	18	18	18	18		36	36				
Ending live weight, kg	133.38^{ab}	129.67^{b}	133.89^{ab}	136.91^{a}	9.07	140.27	126.65	9.01	0.01	< 0.001	0.73
HCW, kg	103.67^{b}	102.20^{b}	105.71^{ab}	107.91^{a}	8.54	110.77	98.98	8.49	0.01	< 0.001	0.74
Chilled side wt, kg	49.99^{b}	49.19^{b}	50.75^{ab}	52.01^{a}	3.78	53.38	47.58	3.76	0.02	< 0.001	0.60
Carcass yield, %	77.72^{b}	78.65^{a}	78.88^{a}	78.80^{a}	1.10	78.88	78.14	1.09	0.02	0.01	0.02
Loin muscle area, cm	53.46	51.22	50.65	52.12	0.85	51.32	52.40	0.60	0.12	0.21	0.89
10th rib back fat depth, cm	1.84^{b}	1.89^{b}	2.26^{a}	2.21^{a}	0.18	2.41	1.69	0.17	< 0.001	< 0.001	< 0.001
Standardized fat-free lean, %[2]	53.28^{a}	53.00^{a}	51.53^{b}	51.75^{b}	0.33	50.78	54.00	0.25	< 0.001	< 0.001	< 0.01
Bone-in cutting yield, %[3]	77.64^{a}	76.70^{b}	75.97^{b}	76.05^{b}	1.10	75.70	77.48	1.09	< 0.001	< 0.001	0.16
Bone-in lean cutting yield, %[4]	62.77^{a}	61.79^{b}	60.96^{bc}	60.67^{c}	1.47	60.47	62.62	1.46	< 0.001	< 0.001	0.11
Boneless cutting yield, %[5]	54.60^{a}	54.09^{ab}	53.32^{c}	53.56^{bc}	0.85	53.23	54.55	0.83	< 0.01	< 0.001	0.46

1 Values based on data collected from heaviest and second heaviest in each pen (142 total pigs)
2 Standardized fat-free lean = ((8.588 + (0.465 x HCW, lb) - (21.896 x fat depth, in) + (3.005 x LTL area, in^2)) / HCW) x 100, (Burson and Berg, 2001)
3 Bone-in cutting yield = [(trimmed ham, kg + bone-in Boston, kg + bone-in picnic, kg + trimmed loin, kg + natural fall belly, kg) / left side chilled weight, kg] × 100.
4 Bone-in lean cutting yield = [(trimmed ham, kg + bone-in Boston butt + bone-in picnic + trimmed loin) / left side chilled weight] × 100.
5 Boneless carcass cutting yield = [((inside ham, kg + outside ham, kg + knuckle, kg + inner shank, kg + lite butt, kg) + (Canadian back loin, kg + tenderloin, kg + sirloin, kg) + (boneless Boston, kg + boneless picnic, kg) + (belly, kg)) / left side chilled weight] × 100.

Carcass Characteristics and Yield

There were no interactions ($P \geq 0.73$) between dietary treatment and sex for ELW, HCW, and LEA. However, interactions between dietary treatment and sex were observed for carcass yield, 10th rib back fat, and standardized fat-free lean (Table 5). Pigs fed DDGS had reduced ($P \leq 0.03$) carcass yields compared with pigs fed the HOSO diets, while pigs fed HOSO diets did not differ ($P > 0.05$) from each other. Despite differences in carcass weights, LEA did not differ ($P = 0.02$) among pigs fed the four dietary treatments. Furthermore, carcasses from pigs fed HOSO4 and HOSO6 measured with greater back fat thickness ($P \leq 0.01$) than carcasses from pigs fed DDGS and HOSO2. Accordingly, carcasses from pigs fed DDGS and HOSO2 had greater ($P \leq 0.01$) standardized fat-free lean than those from pigs fed HOSO4 and HOSO6. Bone-in carcass cutting yield was greater ($P \leq 0.01$) for pigs fed DDGS compared with pigs fed the HOSO dietary treatments. Furthermore, bone-in lean cutting yield was greater ($P \leq 0.01$) for pigs fed DDGS compared with pigs fed HOSO, regardless of inclusion level. However, pigs fed HOSO2 had a greater (*P*

Table 6. Main effects of diet and sex on early chop quality and color[1,2]

	Dietary treatment					Sex			P-value		
Item	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**	**SEM**	**Barrow**	**Gilt**	**SEM**	**Diet**	**Sex**	**Diet × Sex**
Pens, n	18	18	18	18		36	36				
pH[3]	5.61	5.62	5.63	5.62	0.06	5.65	5.59	0.06	0.83	< 0.01	0.52
Visual color[4]	3.49	3.50	3.68	3.61	0.33	3.56	3.58	0.33	0.11	0.67	0.59
Visual marbling[5]	1.71^{b}	1.85^{ab}	2.06^{a}	2.06^{a}	0.36	2.05	1.78	0.35	0.03	< 0.01	0.45
Subjective firmness[6]	2.56	2.47	2.58	2.47	0.10	2.61	2.43	0.07	0.73	0.04	0.75
Lightness[7], L*	51.13	51.31	50.05	51.66	0.77	51.47	50.61	0.63	0.30	0.17	0.23
Redness[8], a*	7.41^{b}	8.17^{a}	8.68^{a}	8.46^{a}	0.72	8.27	8.08	0.71	< 0.001	0.37	0.91
Yellowness[9], b*	4.71	5.10	4.98	5.51	0.66	5.23	4.91	0.63	0.14	0.19	0.09
Drip loss, %	4.04	3.86	4.16	3.48	0.33	3.81	3.95	0.24	0.50	0.67	0.40
Moisture, %	74.16^{a}	73.97^{a}	73.84^{ab}	73.51^{b}	0.48	73.70	74.05	0.47	< 0.01	< 0.01	0.37
Extractable lipid, %	2.40^{b}	2.56^{b}	2.76^{ab}	3.10^{a}	0.16	3.01	2.40	0.13	< 0.01	< 0.001	0.30
Warner-Bratzler shear force[10], kg	3.03	3.02	3.24	3.05	0.10	2.95	3.22	0.07	0.32	< 0.01	0.41
Cook loss[11], %	23.07	23.31	22.89	22.18	0.60	22.50	23.22	0.45	0.53	0.21	0.08

1 Early postmortem traits were evaluated 1 d postmortem
2 Different superscript letters within the same row reflect dietary treatment differences (P ≤ 0.05).
3 Block 1 was measured with MPI and Block 2 was measured with the Hanna Meat pH meter.
4 NPPC color based on the 1999 standards measured in half point increments where 1 = palest, 6 = darkest.
5 NPPC marbling based on the 1999 standards measured in half point increments where 1 = least marbling, 6 = greatest marbling.
6 NPPC firmness based on the 1991 scale measured in half point increments where 1 = softest, 5 = firmest.
7 L* measures darkness (0) to lightness (100; greater L* indicates a lighter color).
8 a* measures redness (greater a* indicates a redder color).
9 b* measures yellowness (greater b* indicates a more yellow color).
10 Warner-Bratzler shear force evaluated on chops cooked to 70°C.
11 Cook loss = [(initial weight, kg - cooked weight, kg) ÷ initial weight, kg] × 100.

Table 7. Main effects of diet and sex on trained taste panel characteristics on a 15-point scale[1]

	Dietary treatment					Sex			*P*-value		
Item	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**	**SEM**	**Barrow**	**Gilt**	**SEM**	**Diet**	**Sex**	**Diet × Sex**
Pens, n	18	18	18	18		36	36				
Tenderness[2]	8.55	8.42	8.47	8.43	0.17	8.54	8.40	0.14	0.86	0.26	0.52
Juiceness[2]	8.27	8.32	8.32	8.24	0.30	8.24	8.33	0.28	0.96	0.50	0.17
Flavor[2]	2.11	2.15	2.12	2.14	0.26	2.12	2.14	0.26	0.75	0.42	0.30
Off-flavors[3]	15	7	9	8		20	19				

[1] Different superscript letters within the same row reflect dietary treatment differences ($P \leq 0.05$).
[2] Sensory scores with a greater value represent a greater degree of tenderness, juiciness, or flavor. Scores were recorded on a 15-point line scale.
[3] Values are reported as the number of noted incidences of off-flavors throughout the panels.

≤ 0.01) bone-in lean cutting yield compared to pigs fed HOSO6, while HOSO4 did not differ ($P > 0.05$) from either extreme.

Loin Quality

There were no interactions ($P \geq 0.08$) between dietary treatment and sex for any fresh quality measurement (Table 7). Ultimate pH did not differ ($P = 0.82$) between pigs fed DDGS and pigs fed the HOSO diets. Visual color and firmness did not differ ($P \geq 0.11$) between pigs fed any dietary treatment; however, visual marbling was increased ($P \leq 0.01$) in loin chops from pigs fed HOSO4 and HOSO6 treatments compared with chops from pigs fed the DDGS diet. Similarly, extractable lipid was greater ($P \leq 0.01$) in fresh loin chops from pigs fed HOSO6 compared with DDGS and HOSO2 diets. Drip loss did not differ ($P = 0.50$) between pigs fed different dietary treatments. Finally, WBSF and cook loss did not differ ($P \geq 0.32$) among pigs fed the four different dietary treatments. No treatment differences ($P \geq 0.75$) were observed in tenderness, juiciness, or flavor of loin chops, as evaluated by trained panelist.

Fat and Belly Quality

Total saturated fatty acid content was different ($P \leq 0.01$) among all dietary treatments with DDGS (33.47%) having the greatest concentration and HOSO6-fed pigs (25.98%) having the least. Total MUFA content of adipose tissue was different ($P \leq 0.01$) between all dietary treatments with pigs fed HOSO6 (61.14%) having the greatest MUFA concentration and DDGS-fed pigs (48.17%) having the least. This effect was the result largely of oleic acid (C18:1*n*-9). The percentage of C18:1*n*-9 was least ($P \leq 0.01$) in pigs fed DDGS (41.31%) and increased with increasing levels of HOSO inclusion. The total PUFA concentration was greater ($P \leq 0.01$) in fat from pigs fed the DDGS diet compared with pigs fed the HOSO diets. However, between the HOSO diets, pigs fed the HOSO2 diet contained a greater ($P \leq 0.01$) concentration of total PUFA than pigs fed the HOSO4 and HOSO6 diets.

Overall, fat from pigs fed DDGS was more saturated than pigs fed the diets containing HOSO as indicated by decreased ($P \leq 0.01$) UFA:SFA ratios. This was likely driven by an increase in the MUFA oleic acid and decrease in the saturated fatty acids palmitic and stearic acid in HOSO diets. However, fat from pigs fed the DDGS diet was also more polyunsaturated than pigs fed the HOSO diets as indicated by increased ($P \leq 0.01$) PUFA:SFA ratios. This degree of unsaturation, however, was not reflected in differences in IV. The IV of fat from pigs fed DDGS was similar to that of pigs fed the HOSO4 diet and reduced ($P \leq 0.01$) compared with pigs fed the HOSO6 diet. Furthermore, pigs fed HOSO2 had a reduced ($P \leq 0.01$) IV compared with pigs fed the other dietary treatments. Bellies from pigs fed DDGS were wider ($P \leq 0.03$) compared with pigs fed HOSO2 and HOSO4, but belly width did not differ ($P > 0.05$) among pigs fed diets containing HOSO (Table 8). Furthermore, bellies were approximately 0.3 cm thinner ($P \leq 0.04$) from pigs fed DDGS compared with pigs fed HOSO

Table 8. Main effects of diet and sex on fresh belly characteristics[1]

	Dietary treatment					Sex			*P*-value		
Item	**DDGS**	**HOSO2**	**HOSO4**	**HOSO6**	**SEM**	**Barrow**	**Gilt**	**SEM**	**Diet**	**Sex**	**Diet × Sex**
Pens, n	18	18	18	18		36	36				
Length, cm	70.81	70.33	70.80	70.49	2.34	71.75	69.47	2.32	0.78	< 0.001	0.94
Width, cm	27.84^{a}	26.20^{b}	26.72^{b}	26.97^{ab}	0.72	27.30	26.56	0.67	0.01	0.04	0.20
Thickness, cm[2]	3.25^{b}	3.46^{a}	3.64^{a}	3.63^{a}	0.28	3.74	3.25	0.27	< 0.001	< 0.001	0.05
Flop, cm	16.14^{b}	22.45^{a}	23.01^{a}	20.18^{a}	4.64	22.91	17.98	4.57	< 0.001	< 0.001	0.10

[1] Different superscript letters within the same row reflect dietary treatment differences ($P \leq 0.05$).
[2] Thickness was an average of measurements from 8 locations from the anterior to posterior, with 4 measurements on each of the dorsal and ventral edges, respectively.

diets, regardless of HOSO inclusion. Similar to belly thickness, belly flop of pigs fed DDGS was decreased ($P \leq 0.02$) compared with pigs fed all levels of HOSO.

Discussion

High oleic soybean oil was hypothesized to serve as an ideal dietary lipid source for pigs due to the significantly increased C18:1 content. The difference in fatty acid composition of the formulated diets confirmed this shift in fatty acid profile. The fatty acid profiles analyzed were consistent with the proportion and type of dietary lipids added to the diet. Quantitatively, oleic acid content was increased in HOSO diets compared to DDGS (15.59 mg/g) diets and increased as increasing levels of HOSO were supplemented (HOSO2 - 27.46 mg/g, HOSO4 - 48.22 mg/g, HOSO6 - 65.64 mg/g). Additionally, there was a quantitative decrease of approximately 10 mg/g of C18:2 in the HOSO dietary treatments compared to the DDGS diet.

Feeding diets containing HOSO generally improved G:F due to reduced ADFI. However, further research conducted with constant ME between the DDGS and HOSO diets could potentially provide insights into differences independent of changes resulting from increased energy intake. Previous studies where dietary oils were added to growing-finishing diets demonstrated increased ME and slowed the movement of feed through the digestion tract reducing feed intake and improving efficiency (NRC, 2012; De la Llata et al., 2001). Inclusion of high-oleic dietary oils, however, does not always result in improved performance. Feeding up to 12% high-oleic sunflower oil to pigs did not alter ADG, ADFI, or G:F when compared with a control sorghum-soybean meal diet (Rhee et al., 1988). While feeding HOSO to growing-finishing swine does have the potential to improve growth performance it will be important to consider the increased costs of diets with the inclusion of HOSO. However, as HOSO becomes more accessible, prices should become more competitive.

Inclusion of HOSO also altered carcass characteristics and, in general, resulted in heavier and fatter carcasses. Overall, backfat increased approximately 0.3 cm for pigs fed HOSO treatments compared with pigs fed DDGS. However, backfat depth at the 10th rib ranged from 1.84 cm to 2.26 cm which still represents an acceptable backfat thickness. As a consequence of increased backfat, calculated standardized fat-free lean and primal weights expressed as a percentage of chilled side weight were reduced by feeding HOSO at 4 or 6% of the diet.

Consumer preferences for fresh pork quality traits vary dramatically depending on background and between countries. The increase in both redness and marbling in loins produced by pigs fed increasing levels of HOSO will potentially have greater appeal for the U.S.'s growing export industry. However, this warrants further investigation as, in the present study, none of the treatment averages would likely qualify for quality-driven export markets. Results of the present study demonstrate HOSO-containing diets had few adverse effects on loin quality including fresh quality, palatability, and color shelf life. Additionally, increased marbling and redness of pork chops from pigs fed the HOSO-supplemented diets suggests HOSO inclusion may have beneficial effects on loin appearance. However, further research including more extensive investigation of flavor profile and oxidative stability on processed products is warranted to fully characterize the potential of feeding HOSO on pork quality.

As expected, the main saturated fatty acid, C16:0, decreased as the inclusion level of HOSO increased. This reduction in saturated fatty acids of pork when feeding increasing levels of HOSO should appeal to the current health-conscious consumer market. In addition to changes in saturated fatty acids, the percentage of oleic acid increased as higher inclusions of HOSO were fed to pigs. However, it is important to be conscious of the potential ramifications of reducing the concentration of saturated fatty acids in pork fat including bacon manufacturing difficulties, oily appearance of products, decreased shelf life, and oxidative damage despite the increase in MUFA (Xu et al., 2010). While fat composition has many quality implications for pork, bacon manufacturing is often cited as one important outcome influenced by fat quality (Xu et al., 2010). Pigs fed the HOSO2 diet consistently produced thicker, firmer bellies that translated into bacon with similar processing yields when compared to pigs fed the DDGS diet. However, as HOSO inclusion levels were increased to 4% and 6%, pigs continued to produce thicker bellies, but had softer fat when compared to pigs fed the diet containing DDGS.

Conclusion

The inclusion of dietary lipids in growing-finishing swine diets has evolved as the swine industry continually adapts to consumer demands and advancements in technology. The addition of dietary lipids to swine diets can provide economic and growth performance benefits. However, understanding the fatty acid composition of dietary feed ingredients for swine is crucial to the impacts it will have on carcass characteristics, pork quality, and shelf life. Overall, feeding HOSO as a dietary feed ingredient to growing-finishing pigs improved growth performance parameters including ADFI and G:F, and resulted in fatter, heavier carcasses. Furthermore, there were no negative implications on pork quality for pigs

fed HOSO and increases in marbling and redness may suggest potential improvements in loin appearance. Finally, feeding HOSO to pigs resulted in an alteration of pork fatty acid composition with bellies having increased oleic acid percentage and decreased palmitic acid and linoleic acid percentages as graded levels of HOSO were added to the diet. As a result, pigs fed the HOSO diets produced thicker bellies with greater flop distances compared to pigs fed DDGS. Ultimately, feeding HOSO as a dietary feed ingredient for pigs has the potential to improve specific growth performance, loin quality, and fresh belly characteristics.

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Nutritional Impact on Mammary Development in Swine

Chantal Farmer
Agriculture and Agri-Food Canada
Sherbrooke R & D Centre, Sherbrooke, QC, Canada J1M 0C8
Phone: 819-780-7222
chantal.farmer@canada.ca

Summary

Sow milk yield is the main determinant of piglet growth rate as it is the only source of energy for suckling piglets. Sows do not produce enough milk to sustain optimal growth of their litter and this problem is made worse with the current use of hyperprolific sow lines. Milk yield is influenced by numerous factors but one factor of importance which is often overlooked is mammary development. In swine, mammary development takes place in prepuberty, as of 3 months of age, during the last third of pregnancy, and during lactation. It is during these periods of rapid mammary growth that nutritional strategies could be used to enhance mammary development. From 90 days of age until puberty, a 20% feed restriction drastically reduces mammary tissue mass whereas decreasing protein intake has no effect on mammary development and feeding the phytoestrogen genistein increases mammary cell number. During late gestation, feeding very high energy levels may have detrimental effects on mammary development and subsequent milk production. On the other hand, increasing concentrations of the growth factor IGF-1 stimulates mammary development. Body condition is important to consider. Gilts that have a lower feed intake during gestation and are too thin at the end of gestation have reduced mammary development. Feeding during lactation also affects mammary development. There is an increase in weight of functional mammary glands when sows are fed more protein or more energy. It is therefore imperative to maximise feed intake of lactating sows. Much remains to be learned about the optimal feeding management to enhance mammary development in gilts and sows.

Introduction

Milk is the main energy source for piglets and is therefore essential for their growth and survival. However, sows cannot produce enough milk to sustain optimal growth of their litters. Indeed, it was shown that ad libitum access to nutrients, achieved via artificial rearing, during the pre-weaning phase results in dramatically heavier weaning weights of piglets compared with sow rearing (Harrell et al., 1993). In that study, artificially-reared pigs weighed 53% more than sow-reared pigs at 21 days of age. More recently, it was also shown that providing supplemental milk to pre-weaning piglets significantly increases their weight at weaning (Miller et al., 2012). The problem of inadequate milk intake by piglets was made even worse with the current use of hyperprolific sows. It is therefore imperative to develop management strategies that will increase sow milk yield. One crucial factor determining sow milking potential is the number of mammary cells that are present at the onset of lactation (Head and Williams, 1991) and more attention should be given to mammary development when developing the best management and nutritional practices for growing replacement gilts and for gestating and lactating sows. Rapid mammary development occurs at three distinctive periods in the life of pigs and it is during these periods that it is possible to attempt to stimulate that development via nutritional or other strategies.

Timing of Mammary Development in Swine

At birth, there is relatively little development of the mammary duct system and mammary glands consist mainly of subcutaneous stromal tissue (Hughes and Varley, 1980). Accumulation of mammary tissue and mammary DNA, which is indicative of cell number, is slow until 90 days of age. The rate of accretion of mammary tissue and DNA then increases four- to sixfold (Sorensen et al., 2002a) so that by the time the gilt is mated, mammary glands are still very small but contain an extensive duct system with numerous budlike outgrowths (Turner, 1952). Puberty has a stimulatory effect on mammogenesis as parenchymal tissue mass (which

contains the milk secreting cells) increases by 51% in gilts that have reached puberty compared with gilts of a similar age that have not started cycling (Farmer et al., 2004).

In pregnant gilts, quantitative development of the mammary glands is slow in the first two-thirds of gestation, while almost all accumulation of mammary tissue takes place in the last third (Hacker and Hill, 1972; Kensinger et al., 1982; Sorensen et al., 2002a). Between days 75 and 112 of gestation, mammary glands undergo major histological changes as the adipose and stromal tissues are extensively replaced by the milk-secreting lobuloalveolar tissue (Hacker and Hill, 1972; Kensinger et al., 1982; Ji et al., 2006). Ji et al. (2006) also reported a shift in mammary gland composition going from a high lipid content, reflective of the extensive adipose in the tissue, to a high protein content during the last third of gestation. The location of the gland on the udder affects its development during gestation. The wet weight of middle glands (3rd, 4th and 5th pairs) is greater than that of posterior glands (6th, 7th and 8th pairs) on both days 102 and 112 of gestation (Ji et al., 2006).

Mammary gland development does not stop at the end of gestation but it continues during lactation. The average weight of suckled mammary glands increases by 57% throughout lactation. This increase is linear going from 381 g on day 5 of lactation to 593 g on day 21 (Kim et al., 1999a). Mammary growth in lactation is affected by the position of the gland on the udder. It is greater for the five more anterior teat pairs compared with the more posterior teat pairs (Kim et al., 2000). Mammary development is also altered by parity. Mammary glands from multiparous sows are heavier than those from primiparous sows at the end of lactation but the increase in mammary gland wet weight between day 113 of gestation and day 26 of lactation is greater for primiparous sows, with values of 63, 21 and 39% for sows of parity 1, 2 and 4, respectively (Beyer et al., 1994). Primiparous sows show an increase in both number and size of mammary cells whereas only an increase in cell size is seen in multiparous sows (Manjarin et al., 2011). Interestingly, it was shown that what happens during lactation in one parity can affect mammary development in the next parity. When a teat is not suckled in parity one, its development will be significantly reduced in parity two, as well as the growth rate of the piglet suckling it (Farmer et al., 2012c). On the other hand, suckling of a teat for only the first two days of lactation in parity one is enough to ensure that its development the following lactation will not be hindered (Farmer et al., 2017a).

Hormonal Impact on Mammary Development

Understanding the impact of various hormones on mammary development is crucial for the development of novel feeding strategies that will enhance mammogenesis. Indeed, certain feeds or feed additives could be used to enhance the secretion of targeted hormones at specific times where they have beneficial effects on the mammary glands. The two most important hormones involved in the control of mammary development in swine are estrogens and prolactin.

Estrogens

The essential role of estrogens is evidenced by the drastic effect of puberty on mammogenesis (Farmer et al., 2004; Sorensen et al., 2006). During gestation, plasma estrogen concentrations increase dramatically after day 75 (DeHoff et al., 1986) and the drastic increase in metabolic activity of the mammary gland is associated with the increase in estrogens of fetal origin. Indeed, there is a relation between mammary DNA and circulating concentrations of estrogen in sows on day 110 of gestation (Kensinger et al. 1986b,c). An earlier study showed that zearalenone, a mycotoxin with estrogen-like activities, affects mammary development. An increase in mammary glandular elements due to ductal hyperplasia was observed in sows receiving zearalenone (Chang et al., 1979). Mammary development was even observed in some of the 7-day old piglets sucking the zearalenone treated sows (Chang et al., 1979). Whether this would have beneficial effects on future lactation performance is not known. The impact of feeding flaxseed on mammary development of gilts was investigated because of its high content of secoisolariciresinol diglycoside, which is a precursor for lignin formation, which in turn exhibits estrogenic activities (Adlercreutz et al., 1987). Yet, dietary supplementation with 10% flaxseed from 88 days until 212 days of age did not lead to significant changes in mammary development on day 212 (Farmer et al., 2007). On the other hand, mammary development was stimulated in growing gilts by providing a dietary source of estrogen. When 2.3 g/day of the phytoestrogen genistein was added to the standard soya diet of gilts from 90 to 183 days of age, there was a 44% increase in mammary parenchymal cells at the end of the treatment period (Farmer et al., 2010).

Prolactin in Growing Gilts

Prolactin is the hormone which has received most attention in terms of its effects on mammary development in swine. The first indication that it may affect mammogenesis came from a trial where prolactin was provided to gilts with the goal of increasing their growth

performance (McLaughlin et al., 1997). No measures of mammary composition were made but visual appraisal suggested a stimulatory effect on mammary development. In a later experiment, growing gilts were injected with 4 mg/day of recombinant porcine prolactin for 29 days starting at 75 kg body weight. This treatment led to a 116% increase in mammary parenchymal tissue mass (Farmer and Palin, 2005). However, mammary secretions were present, suggesting premature onset of lacteal secretions. The potential impact of such a development on future milk needs to be determined.

Prolactin in Gestation

As early as 1945, there were indications that consumption of ergotized barley by late-pregnant sows had a detrimental effect on mammary development. Almost no mammary development was present in sows consuming the ergotized barley in late gestation (Nordskog and Clark, 1945). Furthermore, sows fed ergotized barley showed agalactia. A negative impact of ergots on mammary development when fed for 8 days prior to farrowing was also reported more recently (Kopinski et al., 2007). It is known that there is a prolactin surge associated with parturition in the sow. This peak occurs between approximately 2 days prepartum through several days postpartum (Dusza and Krzymowska, 1981). This is most interesting due to the finding that endotoxins have an inhibitory effect on prolactin secretion during the immediate postpartum period, thereby showing a potential relation between suppression of prolactin and insufficient milk yield in sows (Smith and Wagner, 1984). The first demonstration of the essential role of prolactin for mammary development in pregnant gilts was made over 10 years ago using the dopamine agonist bromocriptine to inhibit prolactin secretion (Farmer et al., 2000). When feeding 10 mg of bromocriptine to gilts thrice daily from days 70 to 110 of gestation, mammary parenchymal tissue mass on day 110 of gestation was 581 g compared with 1011 g for control animals, representing a 42.5% decrease.

It was subsequently shown that the specific time-window where prolactin exerts most of its stimulatory effect on mammary gland growth is from 90 to 109 days of gestation (Farmer and Petitclerc, 2003). Feeding 10 mg of bromocriptine thrice daily to gilts during that specific time period decreased total parenchymal mass by 46% on day 110 of gestation but the treatment had no effect when given from days 50 to 69 or days 70 to 89 of gestation. Recent data showed that when creating a hyperprolactinemic state from days 90 to 110 of gestation, using the dopamine antagonist domperidone, there was a significant beneficial effect on secretory activity of mammary parenchyma and on mammary epithelial cell differentiation (Van Klompenberg et al., 2013). Subsequent milk yield was also improved on days 14 and 21 of lactation, and piglet weight gain until weaning was increased by 21%. Yet, no measures of mammary composition were obtained.

The timing and degree of stimulation of prolactin secretion may be of importance. King et al. (1996) administered high levels of porcine prolactin to first-litter gilts from day 102 of pregnancy through lactation. While concentrations of RNA and DNA in mammary tissue biopsies were not affected by prolactin administration, milk yield was reduced in treated sows. In another study where recombinant porcine prolactin was administered from days 2 to 23 of lactation in third-parity sows, no significant effects on either milk yield or mammary composition were observed (Farmer et al., 1999). This absence of effect was thought to be due to the fact that mammary receptors for prolactin were already saturated in control animals, thereby preventing the exogenous prolactin from having any biological action (Farmer et al., 1999).

Findings up to date therefore suggest that it would be of interest to find ways to increase circulating prolactin concentrations using feed additives that could be used in commercial swine operations. One possibility is the flavonoid plant extract silymarin (from milk-thistle) which increases prolactin concentrations in rats (Capasso et al., 2009) and increases milk yield in women (Di Pierro et al., 2008) and cows (Tedesco et al., 2004). Yet, a recent study showed that even though silymarin increases prolactin concentrations in gestating sows, this increase is not significant enough to have beneficial effects in terms of mammary development (Farmer et al., 2014a). More specifically, 4 g of silymarin was fed twice daily from 90 days until 110 days of gestation, leading to a 51.8% increase in circulating prolactin concentrations 4 days after the onset of treatment. However, this effect was no longer apparent 15 days later. The absence of beneficial effects on mammary development may be due to the fact that prolactin concentrations were not increased enough or for a long enough period of time. It is possible that a larger dose of silymarin could have had a greater effect. Yet, depending on the required duration of treatment, this would most likely not be economically feasible for producers. Silymarin was also fed to lactating sows with no beneficial effects. When 1 or 8 g/d of silymarin was fed throughout a 20 day-lactation, circulating concentrations of prolactin were not increased and piglet growth was not affected (Farmer et al., 2017b).

IGF-1 in Gestation

The insulin-like growth factor-1 (IGF-1) system was shown to be important for normal mammary gland development in mice (Hadsell and Bonnette, 2000). In swine, IGF-1 was suggested to be a local mediator of mammary development in late pregnancy (Simmen et al., 1988). Yet, transgenic over-expression of IGF-1 did not affect sow milk yield (Monaco et al., 2005). Nevertheless, recent results demonstrate that increasing concentrations of IGF-1 via injections of 5 mg/day of porcine somatotropin from days 90 to 109 of gestation stimulates mammary development in gilts (Farmer and Langendijk, 2019). This beneficial effect was both in terms of amount of parenchymal tissue and composition of this tissue. More specifically, there was a 22% increase in parenchymal tissue mass which also contained less dry matter and fat, and more protein, DNA and RNA; hence, suggesting a mammogenic effect of IGF-1

Nutritional Impact on Mammary Development

Feeding Level before Puberty

Nutrition of swine in the growing period or during gestation or lactation can affect mammary development. Feed restriction inhibits mammary development after 90 days of age but not before. A 34% feed restriction of growing gilts from 28 days (weaning) to 90 days of age had no significant impact on mammogenesis (Sorensen et al., 2006), whereas a 20% (Farmer et al., 2004) or 26% (Sorensen et al., 2006) feed restriction from 90 days of age until puberty reduced mammary parenchymal mass by 26.3% and 34.2%, respectively. High feeding levels from 90 days of age until puberty are therefore recommended to ensure optimal mammary development of growing gilts. On the other hand, reducing dietary crude protein from 18.7% to 14.4% in that same period does not affect mammary development (Farmer et al., 2004) suggesting that total feed intake is more important than protein intake per se for mammary development of growing gilts.

Compensatory Feeding

There were early indications that diet deprivation followed by overallowance during the growing, finishing, and gestation phases could be beneficial in terms of milk yield and mammary gene expression in swine (Crenshaw et al., 1989). Yet, later studies could not reproduce those results. Lyvers-Peffer and Rozeboom (2001) investigated the effects of a growth-altering feeding regimen before puberty on mammary development at the end of gestation. They used dietary fiber (35% ground sunflower hulls) to achieve phases of moderate growth which alternated with phases of maximum growth. They reported that gilts on the moderate feeding regimen from 9 to 12 weeks and 15 to 20 weeks of age had less mammary parenchyma on day 110 of gestation than control gilts. In a later experiment using a similar approach, specific periods of diet deprivation (providing 70% of the protein and DE contents from the control diet) followed by over-allowance (providing 115% of the protein and DE contents from the control diet) in growing gilts did not have any beneficial effect on mammary development after puberty. In fact, this feeding regime led to a decrease in parenchymal tissue mass at puberty (Farmer et al., 2012a). The same nutritional treatment also did not affect parenchymal mass at the end of gestation but led to a tendency for reduced percent protein in mammary parenchyma (Farmer et al., 2012b). It is important to note that in those latter studies by Farmer et al. (2012ab), no compensatory growth was observed in gilts at the end of the finishing period. Even though formulation of the restriction diet decreased the DE content by 30% compared to that of the control diet in both Crenshaw and Farmer's studies, the composition of the fiber fraction differed, which may have led to the discrepancy in results. Sunflower hulls were used as fiber source by Crenshaw et al. (1989; J. D. Crenshaw, APC, Inc., Ankeny, IA, personal communication), whereas soybean hulls and wheat middlings were used by Farmer et al. (2012ab). It is therefore still not known whether a compensatory feeding regime in the growing-finishing period only could stimulate mammary development in gilts.

Feeding Level in Gestation

Nutrition during pregnancy does affect mammary development at the end of gestation. An early study where body composition of sows was altered by manipulating protein and energy intakes during gestation demonstrated that overly fat (36 mm backfat) and leaner gilts (24 mm backfat) had similar mammary weights at the end of gestation, but that there was a drastic reduction (approximately threefold) in mammary DNA concentration (i.e., cell number) in overly fat gilts (Head and Williams, 1991). Yet, these body conditions are not representative of what is seen commercially and a project was therefore carried out to establish if differences in body fat that are commonly seen can lead to changes in mammary development at the end of gestation. Gilts of similar body weight at mating were fed different amounts of feed throughout gestation (1.30-, 1.58-, or 1.82-times maintenance requirements) to achieve three levels of body fat, namely, 12-15 (lean), 17-19 (medium) and 21-26 (fat) mm on day 109 of gestation. Parenchymal tissue mass was significantly reduced in lean gilts, being 1059, 1370 and 1444 g for lean, medium and fat

gilts, respectively (Farmer et al., 2016a). It is therefore apparent that being too thin at the end of gestation is detrimental for mammary development. On the other hand, if body conditions differ at the end of gestation because of innate differences already present at mating, results differ. Indeed, when backfat differences were present at mating and were maintained during gestation through varying feeding levels, parenchymal tissue mass at the end of gestation was not affected but its composition was altered (Farmer et al., 2016b). Thinner gilts (12-15 mm backfat) had greater concentrations of protein, DNA (i.e. cell number) and RNA (i.e. metabolic activity) in their mammary parenchyma compared with gilts from the two other groups. Taken together, these studies highlight that feeding during gestation is most important in terms of mammary development and that one should avoid underfeeding to ensure maximal amount of parenchymal tissue mass.

When using a period of diet deprivation (providing 70% of the protein and DE contents from the control diet) for the first 10 weeks of gestation, followed by a period of over-allowance (providing 115% of the protein and DE contents from the control diet) until the end of gestation, there was less parenchymal tissue at the end of gestation with no changes in parenchymal tissue composition in treated gilts (Farmer et al., 2014b). The goal of that project was to look at the effect of compensatory feeding on mammary development yet, even though growth rate was increased in the overfeeding period, this increase was not large enough to compensate for the body weight loss in the restriction period during early gestation. A better adapted feeding regime needs to be developed to be able to truly assess the impact of compensatory feeding on mammary development of gestating gilts.

Feed Composition in Gestation

Not only amount of feed but also composition of feed during gestation can be important for mammary development. Increasing dietary energy (5.76 vs. 10.5 Mcal/ME) from day 75 of gestation until the end of gestation decreased mammary parenchymal weight and parenchymal DNA on day 105 of gestation (Weldon et al., 1991). On the other hand, increasing protein intake (330 vs. 216 g CP/day) had no effect on mammary development (Weldon et al., 1991). This finding was later corroborated by Kusina et al. (1999) who showed that lysine intakes of 4, 8 or 16 g/day from days 25 to 105 of gestation did not alter mammary development at the end of gestation. However, subsequent milk yield was greater in sows fed 16 g/day of lysine. Recent data showed increased average daily gains of suckling piglets when sows were fed 20.6 instead of 14.7 g/day of lysine from day 90 of gestation until farrowing (Che et al., 2019), and it was suggested that this could be due to enhanced mammogenesis. Hence, the role of lysine on mammary development in late pregnancy needs to be further investigated.

A recent report indicated that nutrition of sows in gestation and lactation can affect mammary development of their offspring. Indeed, dietary supplementation with 10% flaxseed from day 63 of gestation until the end of lactation increased mammary parenchymal mass of the offspring at puberty (Farmer and Palin, 2008). This was a first demonstration of such an in utero effect in swine and it leads to new avenues in terms of development of feeding strategies to enhance mammogenesis.

Feed Intake in Lactation

Nutrition during the last phase of rapid mammary accretion, namely lactation, also affects mammary development, yet there is very little information on the subject. It was shown that both increased energy and protein intakes stimulate mammary gland growth. Kim et al. (1999b) fed lactating primiparous sows 4 diets that were a combination of different protein (32 or 65 g lysine/day) and energy (12 or 17.5 Mcal ME/day) levels. Wet and dry weights of suckled mammary glands were positively affected by both energy and protein intakes. Results showed that wet and dry mammary weights were maximized when sows consumed an average of 16.5 Mcal of ME and 950 g of crude protein per day, the latter being equivalent to 52.3 g of lysine daily. It was suggested that lactating sows may need slightly more lysine than the currently recommended amount in order to achieve maximal mammary gland growth during lactation. Litter size positively affects mammary development and it was estimated that lactating sows should be fed an additional 0.96 g of lysine per day for each additional piglet in a litter of 6 and above (Kim et al., 1999c). It is apparent from published findings that nutrient intake during lactation is important for mammary development, thereby supporting the current recommendation to increase sow feed intake as much as possible during lactation to maximize milk yield.

Conclusion

Mammary development in swine can be altered by many factors, one of them being the nutritional strategy used in the prepubertal, gestation and lactation phases of production. Table 1 summarizes the various nutritional treatments that can affect mammary development in swine. In growing gilts, feed restriction as of 90 days of age hinders mammary development and

Table 1. Nutritional treatments that stimulated mammary development (in terms of mass of parenchymal tissue or amount of parenchymal DNA) in pigs (from Farmer, 2018).

Treatment	Period[1]	Effect on parenchyma	References
10% flaxseed	*In utero* (day 63 gestation to end lactation)	31% parenchymal weight	Farmer et al. (2007)
2.3 g/d of genistein	90 to 183 days	44% total DNA	Farmer et al. (2010)
Ad libitum feeding vs. 25% feed restriction	90 days to puberty	46% parenchymal weight	Sorensen et al. (2002b)
Ad libitum feeding vs. 20% feed restriction	90 days to puberty	36% parenchymal weight	Farmer et al. (2004)
Ad libitum feeding vs. 33% feed restriction	90 days to 5½ months	52% parenchymal weight 28% total DNA	Sorensen et al. (2006)
24 vs. 36 mm BF[2] at end of gestation via changes in energy and protein intakes	Gestation	approximately 240% DNA concentrations	Head and Williams (1991)
21 to 26 or 17 to 19 mm BF vs. 12 to 15 mm BF at end of gestation via changes in feed intake	Gestation	average of 33% parenchymal weight	Farmer et al. (2016a)
5.76 vs. 10.5 Mcal ME/d	Day 75 to end of gestation	27% parenchymal weight	Weldon et al. (1991)
Domperidone (0.4 mg /kg BW)	Days 90 to 110 of gestation[3]	80% in lumen diameter of mammary epithelial cells	Van Klompenberg et al. (2013)
17.5 vs. 12 Mcal ME/d	Lactation	parenchymal weight[4]	Kim et al. (1999b)
65 vs. 32 g lysine/d	Lactation	parenchymal weight[4]	Kim et al. (1999b)

[1] Period where treatment was imposed.
[2] BF = backfat.
[3] Mammary development was not measured but there was an increase in mammary epithelial cell differentiation.
[4] Percent increase could not be determined from the published data.

there are indications that supplying the phytoestrogen genistein or increasing concentrations of prolactin from 90 days to puberty stimulates mammogenesis. Nutritional studies in gestation showed negative effects of overfeeding energy to achieve obesity (36 mm backfat) and also showed nefarious effects of a gilt being underfed and having less than 16 mm backfat on mammary development on day 110 of gestation. When using feed restriction followed by over-feeding in gestation, mammary parenchymal mass was reduced. Yet, the effect of a greater compensatory growth during late gestation on mammary development still needs to be determined. Mammary gland growth in lactation responds to increased protein and energy intakes indicating the importance of nutrient intake for mammary development. Special attention must be given to lysine as lactating sows may need slightly more than currently recommended. Furthermore, litter size must be taken into consideration as it affects mammary development and lysine needs are increased in a litter of 6 or more piglets. It is obvious from our current state of knowledge that much still remains to be learned in order to develop the best nutritional strategies for replacement gilts, gestating gilts, and lactating sows in order to maximize their mammary development, hence, their lactation performance.

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Supplemental Yeast Fermentation Products Effect on Sow Lactation Performance and Post-Partum Recovery Based on Uterine Fluids and Blood Parameters

Ricardo M. Garcia†, Morgan T. Thayer†, Kayla M. Mills†, Jacob A. Richert†, Derek B. Petry¶, John S. Radcliffe†, Vinícius S. Cantarelli*, and Brian T. Richert†2

†Animal Science Department, Purdue University, West Lafayette, IN, 47907-2041

¶Diamond V, Cedar Rapids, IA, 52404

*Animal Science Department, Universidade Federal de Lavras, Lavras, Minas Gerais, 37200-000, Brazil.

Phone: 765-494-4837

brichert @purdue.edu

Summary

Sow parturition is a stressful critical life event for both the sow and the piglet. The effect of nutrition preceding and post-partum to impact sow recovery and future lactation performance is an intriguing, difficult area to research. This is partially because of the difficulty in individual sow variability in age, size, body condition, birthing litter sizes, potential dystocia issues, metabolic states, etc. The parturition and recovery process also involves large immune and stress responses. The question addressed in this study is whether the feeding of Saccharomyces cerevisia fermentation products (SCFP) prior to parturition and throughout lactation can impact this process and improve sow and litter performance. A developmental oral liquid fermentation product that was delivered daily sped the recovery of the sow post-partum, increasing sow feed intake by day 4 post-partum and both the dry in-feed and liquid products increasing feed intake week 2 and 3 of lactation (13-19% overall), resulting in 2.5-3.2 kg increases in litter weaning weights and reduced percentage of light weight pigs weaned under 3.5 kg. Both SCFP products tended to increased sow serum IL-8, while the dry product tended to reduce sow blood IL-4 and IL-10 days 2 and 6 post-partum. The liquid product increased uterine flush concentration of TNF-alpha and INF-gamma. The use of nutritional supplements like these SCFP products that can potentially impact the sow's immune response and speed her recovery deserve further research attention, finding roles and uses to improve sow performance and welfare.

Introduction

Recent improvements in sow lactation performance through genetic selection, advanced nutrition, and improved reproductive technologies have increased litter sizes to 13-15+, increasing parturition and lactation stress in meeting these new productivity norms. Improving sow feed intake is a critical piece when trying to meet these metabolic demands, as sows with greater feed intake are likely to produce more milk (Strathe et al., 2017) to help meet these large litter demands. Formulating a well-balanced diet is crucial to allow sows to express their genetic potential but voluntary feed intake of lactating sows can become a challenge in situations like heat stress (Bjerg et al. 2020; Silva et al., 2021), lameness (Heinonen et al., 2013), sickness, and over conditioned gestating sows (Eissen et al., 2000). Taken together, low feed intake and excessive mobilization of body reserves during lactation can negatively affect both litter growth and subsequent sow reproductive performance (Stathe et al., 2017).

In the mid and late gestation periods, hyperprolific sows develop a pro-inflammatory status (Liu et al., 2021; Cheng et al., 2018) with increasing systemic oxidative stress (Berchieri-Ronchi et al., 2011, Chen et al., 2020) as the sow prepares for the onset of the farrowing processes (Jabbour et al., 2009). Among many factors that can affect feed intake, inflammatory mediators can have an important role not only lowering the appetite but also modifying the partitioning of nutrients (Sauber et al., 1999). This inflammation may increase the animal's maintenance requirements, reduce milk production and piglet growth, and impact reproductive performance. Yeast fermentation products, classified as

postbiotics, contain soluble compounds from yeast cells after their lysis as well as the medium of fermentation (Aguilar-Toalá et al., 2018). Yeast products and derivates are known to have positive effects on swine intestinal health and growth performance acting as prebiotics (Kumar et al., 2017; Price et al., 2010) and producing immunomodulatory effects (Sanchez et al., 2018 (a); Sanchez et al., 2018(b); Zanello et al., 2013). Relief of inflammatory status of sows post-farrowing may promote greater feed intake in the early stage of lactation and consequently help mitigate the effects of inflammatory processes after parturition. Therefore, the objective of this study was to evaluate the effects of *Saccharomyces cerevisiae* fermentation products (**SCFP**) on lactating sows and offspring performance.

Materials and Methods

All experimental procedures and animal housing were reviewed and approved by the Purdue University Animal Care and Use Committee. In general, animal care followed the Guide for Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Sciences Societies, 2010). The experiment was performed in the Animal Sciences Research and Education Center (**ASREC**) at Purdue University. The two SCFP products tested were provided by Diamond V (Cedar Rapids, IA, USA) and contain a variety of compounds from the fermentation processes such as mannan-oligosaccharides, β-glucans, yeast metabolites, yeast cell wall fragments as well as the media utilized in the fermentation. The two SCFP products were a liquid developmental product (**LIQP**) that was provided orally with a sheep dosing gun and a dry product (**DRY**; **XPC**) added to the feed at the expense of corn in the control diet.

Animals and Treatments

Pregnant sows (Landrace x Yorkshire; n = 140; avg parity 3.1 ± 0.35) were blocked by parity and body condition score approximately on day 112 of gestation and allotted to one of four treatments: 1) Control diet (corn-soybean meal basal diet; CON), 2) CON + 15 mL of LIQP from d 112 to weaning (**LIQ**), 3) CON + 0.20% of XPC from d 112 to weaning (**DRY**), and 4) DRY + 15 mL of LIQP from d 112 to d 7 post-farrowing (L+D). Sows were fed a common 30% DDGS gestation diet until d 111 of pregnancy and then were moved to the farrowing barn and fed dietary treatments on day 112 (Table 1). Lactation diets were formulated to meet or exceed the nutrient requirements (NRC, 2012). Farrowing stalls were 0.5 x 2.1 m with one stainless steel feeder and stainless steel water drinker. The farrowing pens were 1.52 x 2.64 m and the creep space for the piglets was 0.46 m wide on each side of the sow stalls. All sows were allowed to eat 3 kg/day upon entry until farrowing and *ad libitum* after farrowing until weaning, with

Table 1. Lactation diet composition.

Ingredients, %	CON	DRY
Corn	50.303	50.103
Soybean meal, 47.5% CP	32.600	32.600
Dried distillers grain with solubles	10.000	10.000
Swine grease	3.000	3.000
Limestone	1.430	1.430
Monocalcium phosphate	1.350	1.350
Vitamin premix[1]	0.250	0.250
Sow premix[2]	0.250	0.250
Trace mineral premix[3]	0.125	0.125
Se premix[4]	0.050	0.050
Phytase	0.100	0.100
Salt	0.500	0.500
Availa Zn 120[5]	0.042	0.042
XPC[6]	0.000	0.200
Calculated Composition		
ME, kcal/kg	3353.30	3353.30
Crude protein, %	22.33	22.33
Total Lysine, %	1.183	1.183
SID[7] Lys, %	1.001	1.001
SID Met, %	0.311	0.311
SID Met+Cys, %	0.615	0.615
SID Thr, %	0.693	0.693
SID Tryp, %	0.233	0.233
SID Iso, %	0.809	0.809
SID Val, %	0.889	0.889
Calcium, %	0.902	0.902
Total P, %	0.732	0.732
ATTD P, %	0.501	0.501
Analyzed Composition*		
Crude Protein, %	20.52	21.07
Crude Fiber, %	2.84	2.85
Crude Fat, %	5.12	5.58
NDF, %	9.31	9.42
ADF, %	4.56	4.87
Calcium, %	1.03	1.19
Total P, %	0.665	0.716
Lysine, %	1.10	1.11
Methionine, %	0.29	0.31
Cysteine, %	0.31	0.33
Threonine, %	0.74	0.74
Tryptophan, %	0.25	0.26
Isoleucine, %	0.93	0.94
Leucine, %	1.81	1.82
Valine, %	1.06	1.06

1 Provided per kg of diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 44.1 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.1 mg and B_{12} 38.6 µg.
2 Provided per kg of diet: biotin, 0.22 mg; folic acid, 1.65 mg; choline, 16 mg; pyridoxine, 4.96 mg; vitamin E, 22 IU; chromium, 0.20 mg; and carnitine, 49.6 mg.
3 Provided per kg of diet: Fe, 121.3 mg; Zn, 121.2 mg; Mn, 15.0 mg; Cu, 11.3 mg; and I, 0.46 mg.
4 Provided 0.3 ppm Se.
5 Provided per kg of diet: 120 ppm of zinc.
6 *Saccaromyches cerevisiae* fermentation product (Diamond V, Cedar Rapids, IA, USA).
7 Standardized ileal-digestible
*Analysis conducted by University of Missouri Experiment Station Chemical Laboratories.

feeders evaluated three times per day (0700, 1200, and 1600 h). Feed left over was weighed every morning before the first feeding to calculate daily feed intake. Each crate was equipped with a water meter (Assured Automation, Roselle, NJ, model WM-PD50) and daily water intake (**DWI**) until d 7 of lactation and weekly average DWI (**ADWI**) were calculated.

Animal Handling and Measurements

Sows were weighed on day 112 of gestation and day 1, 7 and weaning of lactation (IQ plus 355-2A; Rice Lake, WI, USA). Back fat (**BF**) and loin depth (**LD**) were evaluated on day 112 of gestation and on weaning day using ultrasound (Aloka SSD 500V, Aloka Co., Ltd., Tokyo, Japan). The BF and LD measurements were performed from the last rib forward, 6.5 cm away from the vertebral column (P2 point), on each side to calculate the means. Sows farrowed either naturally or were induced on day 115 of gestation (2 cc prostaglandin; Lutalyse®, Zoetis Inc., Parsippany-Troy Hills, NJ, U.S.A). Farrowing was monitored and assistance were given when birth interval exceeded 40 min. The reproductive performance was evaluated as the number of total piglets born, piglets born alive, stillborn piglets, and mummified fetuses.

Piglets were weighed on day 1 (before cross-fostering) using a calibrated scale (RL-DBS-2; Rice Lake, WI, U.S.A), on day 7 and weaning day using a calibrated scale (IQ plus 390-DC; Rice Lake, WI, U.S.A) to calculate individual pig and litter ADG. After 24 h of birth and up to 48 h, litters were cross-fostered within the same treatments to equalize litter size according to the number of functional teats/sow. Piglet processing was performed on day 1 and included receiving supplemental iron injection (2 cc, Uniferon 200, Pharmacosmos A/S, Holbaek, Denmark), ear notching, tail docking, teeth clipping, and castration for males. All pigs were weaned at the same day (18.1 ±0.30 days of age).

Immunological Parameters

Colostrum samples (50 mL/sow) were collected from the front, middle, and back teats within 8 h of the first pig born to estimate immunoglobulin concentration using a Brix refractometer (Misco PA201, Misco, Solon, OH, U.S.A). The average of three readings were used to calculate the Brix value.

Blood samples (2 pigs/litter) were collected from a jugular vein 24 h after birth in EDTA tubes (1 mL/pig). The plasma obtained after centrifugation (2000 x g for 10 min; E8 Centrifuge, LW Scientific Inc, Lawrenceville, GA, U.S.A) was aliquoted in three parts, two parts were stored at -80 C for immunoglobulins analysis, and the third part was used immediately for immunocrit ratio analysis. Immunoglobulins (IgA and IgG) were analyzed using a commercially available ELISA kit (Eagle Bioscience, Inc., Nashua, NH) according to the manufacturer's instructions. The immunocrit ratio was calculated by adding 50 µL of ammonium sulfate [40%; (NH4)2SO4] to 50 µL of plasma. The mixture was used to fill a hematocrit microcapillary tub that was centrifugated for 10 min using a micro-hematocrit centrifuge (14800 x g; LWS M24, LW Scientific Inc, Lawrenceville, GA, U.S.A). Then, the microcapillary tubes, in triplicates per pig, were placed in a microhematocrit reader (EZ Reader, LW Scientific Inc, Lawrenceville, GA, U.S.A) to get a direct reading of the ratio between the length of the mixture and the length of the precipitated immunoglobulins.

Interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were quantified from serum samples collected on d 112 of gestation, d 2 and 6 post-farrowing, and from uterine fluid collected on d 2, 4, and 6 post-farrowing on 10 sows/treatment.

Wean to Estrus Interval

After weaning, sows were moved to individual gestation crates (0.6 x 2.3 m) and fed a common gestation diet (2.3 kg/day). Twice each day (0900 and 1500), sows were checked for estrus signs by back pressure test and exposure to a mature boar. The wean to estrus interval (**WEI**) was recorded. Sows were kept in individual crates up to day 30 of gestation and checked for pregnancy using ultrasound before transferring to a group housing system.

Statistical Analysis

All variables measured were tested for normality using Shapiro-Wilk test before analysis, and any variable that failed to follow a normal distribution was transformed through the RANK procedure in SAS (SAS Inst. Inc., Cary, NC). The sow and its litter were considered the experimental unit. Data were analyzed using the PROCMIXED procedure in SAS (SAS Institute Inc., Cary, NC, USA). Pre-planned orthogonal contrasts were used to compare the different sources of SCFP to the control and each other. The daily water and feed intake in the first week of lactation were analyzed as repeated measurements. All data are reported as least squares means and SEM. Means were considered significant different when $P \leq 0.05$ and trends were discussed when $0.05 < P \leq 0.10$.

Results

Sows had similar BW (P > 0.13), BF (P > 0.74), and LD (P > 0.40) at the beginning of the experimental period (Table 2). The sow BW at weaning was 5% greater for SCFP treatments compared to CON (P = 0.03) and LIQ sows tended to be 4% heavier than CON (P = 0.09) but no difference between LIQ and DRY was detected (P = 0.82). Sows from LIQ group had less BW losses (4.9%) throughout the lactation period compared to CON (7.2%; P = 0.05) and to DRY (6.8%; P = 0.09). No difference among treatments was observed in the final back fat thickness and loin depth (P > 0.19) as well as in the overall change in both tissues (P > 0.24).

The ADFI tended to be greater for SCFP treatments compared to CON (P = 0.057; Table 3) and LIQ sows had greater ADFI than CON (P < 0.01) and tended to have greater ADFI than DRY (P = 0.09) in the first week of lactation (Table 3). In the second and third weeks of lactation, as well as for the overall lactation period, SCFP treatments had greater ADFI than CON (P < 0.01), LIQ sows had greater ADFI then CON and DRY (P ≤ 0.04). Weekly as well overall average daily water intake did not differ among treatments (P > 0.17; Table 4).

The number of total born, born alive, stillborn, mummified fetus, the number of pigs sows were allowed to nurse after cross-fostering, and the number of pigs weaned per sow did not differ among treatments (P > 0.15; Table 5). Litter weaning weights were not different among treatments (P > 0.19), however litter gain approached a trend (P = 0.131) for the SCFP to improve

Table 2. Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sows (n = 140) body weights.

	Treatments[1]					Contrast *P*-value[2]		
Performance	**CON**	**LIQ**	**DRY**	**L+D**	**SEM**	**1**	**2**	**3**
Parity	3.1	3.2	3.1	3.0	0.35	0.90	0.94	0.88
Lactation length, days	18.1	18.0	17.9	18.2	0.289	0.91	0.82	0.86
Initial BW (d 1), kg	247.5	252.0	259.0	256.0	10.36	0.13	0.49	0.28
Final BW (weaning), kg	230.2	240.3	241.7	240.5	10.48	0.03	0.09	0.82
Overall BW change, %	-7.2	-4.9	-6.8	-6.6	0.98	0.30	0.05	0.09

1 Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

2 Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.

Table 3. Effect of Saccharomyces cerevisiae fermentation product (SCFP) on lactating sows (n = 140) average daily feed intake.

	Treatments[1]					Contrast *P*-value[2]		
Feed Intake, kg	**CON**	**LIQ**	**DRY**	**L+D**	**SEM**	**1**	**2**	**3**
Gestation period								
d 112, kg	3.10	3.20	3.01	3.11	0.067	0.88	0.30	0.06
d 113, kg	3.18	3.14	3.17	3.13	0.088	0.75	0.66	0.66
d 114, kg	2.86	3.16	2.93	3.17	0.162	0.34	0.20	0.25
d 115 (farrowing day), kg	2.69	3.07	3.15	3.06	0.340	0.17	0.33	0.74
Lactation period								
d 1 – 7, kg	4.33	5.08	4.62	4.58	0.281	0.06	0.007	0.09
d 8 - 14, kg	5.60	6.65	6.03	6.21	0.358	0.005	< 0.001	0.04
d 15 - weaning, kg	5.45	6.57	5.91	6.37	0.284	0.002	< 0.001	0.03
Overall, kg	5.05	6.03	5.46	5.62	0.276	0.002	< 0.001	0.03

1 Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

2 Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.

Table 4. Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sows (n = 140) average daily water intake.

	Treatments[1]					Contrast P-value[2]		
Water intake, L/day	**CON**	**LIQ**	**DRY**	**L+D**	**SEM**	**1**	**2**	**3**
Gestation period								
d 112, L/day	20.24	20.30	18.81	16.65	2.651	0.69	0.96	0.74
d 113, L/day	25.89	19.93	22.15	19.43	2.836	0.17	0.18	0.55
d 114, L/day	24.02	20.80	20.40	20.51	2.821	0.36	0.40	0.93
d 115 (farrowing day), L/day	17.28	15.50	16.08	13.34	2.565	0.18	0.42	0.86
Lactation period								
d 1 - 7, L/day	22.25	23.80	22.84	20.58	1.876	0.96	0.65	0.65
d 8 - 14, L/day	32.73	31.79	33.62	29.07	2.315	0.54	0.63	0.40
d 15 - weaning, L/day	37.04	34.18	35.05	28.90	3.715	0.34	0.59	0.51
Overall, L/day	30.06	29.33	34.30	26.99	3.032	0.75	0.84	0.43

1 Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

2 Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.

litter weight gains over the control. Litter ADG in the first week of lactation was not affected by treatments ($P > 0.45$) but litters from SCFP treatments had greater ADG than CON from d 8 until weaning ($P = 0.040$) and tended to have greater ADG than CON in the overall lactation period ($P = 0.099$). Individual pig weights were not affected by treatments on d 1 or d 7 of life ($P > 0.13$). At weaning, pigs from LIQ sows tended to be 5.5% heavier than pigs weaned from CON sows ($P = 0.098$) but did not differ from DRY ($P = 0.318$). Pigs weaned from sows fed SCFP treatments had greater ADG than CON ($P = 0.047$) while pigs from LIQ sows tended to have greater ADG than CON ($P = 0.087$) and did not differ from pigs of DRY sows ($P = 0.610$). The proportion of pigs weaned lighter than 3.5 kg from sows fed SCFP treatments ($P = 0.077$) or sows fed LIQ ($P = 0.104$) tended to be lower the CON. The pre-weaning mortality rates in the first week as well as the overall lactation period were not affected by treatments ($P > 0.50$).

Table 5. Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sows (n = 140) and offspring performance.

	Treatments[1]					Contrast *P*-value[2]		
Item	**CON**	**LIQ**	**DRY**	**L+D**	**SEM**	**1**	**2**	**3**
Total born	13.1	12.6	13.3	12.4	0.59	0.81	0.70	0.45
Born alive	12.0	11.7	12.2	11.5	0.56	0.96	0.81	0.55
Number after cross-fostering	11.9	11.7	11.9	11.8	0.24	0.68	0.48	0.50
Weaned, n	10.3	10.3	10.6	10.4	0.23	0.58	0.86	0.44
Litter weight at weaning, kg	60.1	63.2	62.6	63.3	1.94	0.20	0.26	0.63
Litter gain, kg	44.0	46.1	46.0	47.4	1.61	0.13	0.29	0.94
Litter ADG d 8 -weaning, kg	2.84	3.01	3.03	3.10	0.084	0.04	0.14	0.99
Litter ADG, kg	2.43	2.55	2.58	2.60	0.069	0.10	0.26	0.85
Pig weight d 1, kg	1.54	1.62	1.52	1.50	0.041	0.85	0.14	0.08
Pig weight at weaning, kg	5.82	6.14	5.95	6.11	0.157	0.12	0.10	0.32
Pig ADG d1-weaning, g	238	251	247	253	6.3	0.05	0.09	0.61
PBW[3] at weaning < 3.5 kg, %	5.4	2.0	2.5	2.9	1.91	0.08	0.10	0.79
d 1 -7 mortality, %	9.8	9.8	9.6	9.7	1.80	0.86	0.94	0.98
d 1- weaning mortality, %	13.7	11.4	11.0	12.3	2.04	0.51	0.86	0.50

1 Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
2 Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.
3 PBW: pig body weight.

Table 6. Effect of *Saccharomyces cerevisiae* fermentation product (SC) sources for lactating sows (n = 40) on colostrum quality and piglet immunoglobulins.

	Treatments[1]					Contrast *P*-value[2]		
Item	**CON**	**LIQ**	**DRY**	**L+D**	**SEM**	**1**	**2**	**3**
Plasma IgG, mg/mL	23.24	21.85	20.10	23.49	1.243	0.21	0.32	0.21
Plasma IgA, mg/mL	8.04	7.74	7.89	6.56	0.779	0.35	0.74	0.87
Immunocrit ratio[3]	0.20	0.19	0.19	0.19	0.014	0.56	0.49	0.72
Brix value[4]	25.98	26.32	26.09	25.79	0.473	0.96	0.77	0.84

1 Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
2 Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.
3 Serum obtained from two piglets/litter was analyzed for immunoglobulins and immunocrit ratio by adding 40% $(NH_4)_2SO_4$ and measuring the length of the precipitate as an estimation of total immunoglobulins present in the serum.
4 Colostrum immunoglobulin concentration was estimated by using Brix refractometer between 1-8 h post-farrowing of the first pig of the litter.

No effects of treatments were observed in the sow colostrum Brix values ($P > 0.77$; Table 6) nor in the pig plasma immunological parameters IgG, IgA, and immunocrit ratio ($P > 0.21$). The sows fed the LIQ and D+L treatments had the greatest serum IL-10 concentration ($P < 0.001$) and sows fed CON tended to have lower concentration of IL-8 ($P < 0.06$) vs. other treatments (data not shown). In the uterine fluid, LIQ and D+L sows had greater INF-γ ($P = 0.04$) concentrations and CON tended to have the least concentration of TNF-α ($P = 0.08$).

The average daily follicle growth post-weaning was greater for SCFP treatments than CON ($P = 0.05$; Table 7) and tended to be greater in sows fed LIQ than CON ($P = 0.09$) but did not differ between LIQ and DRY ($P = 0.99$). The wean to estrus interval was 0.5 day shorter in sows fed LIQ than CON ($P < 0.01$) and 0.9 day shorter than DRY ($P < 0.01$).

Discussion

In the present study, SCFP were fed to sows pre- and post-farrowing aiming for a better parturition recovery and lactation performance. Less BW losses were observed only in sows fed LIQ even though all SCFP groups had greater sow BW at weaning. The lactation

Table 7. Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sows (n = 140) subsequent follicle growth and wean-to-estrus interval.

	Treatments[1]					Contrast *P*-value[2]		
Item[3]	CON	LIQ	DRY	L+D	SEM	1	2	3
Avg. daily follicle growth[4], mm	0.28	0.41	0.40	0.38	0.057	0.05	0.09	0.96
Wean to estrus interval, days	4.9	4.4	5.3	4.9	0.17	0.43	0.006	< 0.001

1 Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
2 Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.
3 Follicles were measured every 12 hours from day two after weaning until ovulation.
4 Calculated as the difference between size prior to ovulation and size at d 2 divided by number of days from wean to ovulation.

feed intake was improved (13%) by SCFP and markedly greater (19%) in sows fed the LIQ source of yeast derivates. For sows fed the LIQ product, this increase in sow feed intake started as early as 4 days post-partum. Shen et al. (2011) evaluated the same dried version of SCFP used in this study for gestating and lactating sows and showed that litter BW gain was improved, possibly through increased milk production, while not affecting colostrum and milk nutrient composition. Our results showed that possibly due to the greater feed intake, sows had increased milk production (not measured) and litter performance, as shown by the greater litter and individual pig ADG from sows fed any of the SCFP tested. The litter BW gain was improved in other studies although the voluntary feed intake was not affected by feeding yeast cultures to lactating sows (Shen et al., 2011; Kim et al., 2008; Kim et al., 2010). Indeed, it was observed that the litter ADG after the first week until weaning was significantly greater for sows fed SCFP. It is possible to infer that those sows fed SCFP had a quicker recover post-farrowing based on the shift in immune cytokines in the blood and uterine fluid, improving their capacity to eat and support litter growth. Moreover, the greatest effect of SCFP on feed intake observed in sows fed the liquid source allowed them to wean heavier pigs than CON sows. To our knowledge, this is the first SCFP in a liquid form tested in sows. We speculate that some of the benefits over the dried source is due to the fixed amount given daily instead of variable amount when mixed in the feed, which ultimately varies according to the daily feed intake, especially around the time of farrowing.

Several nutritional strategies to increase colostrum and milk quality and output have been reported in the literature. Feed additives have also demonstrated to benefit milk production and litter growth. Ho et al. (2020) have shown that chitosan oligosaccharide fed to gilts improved milk yield, litter growth, and the concentration of serum immunoglobulins in the offspring. Our results showed that the colostrum quality, measured by the Brix value and piglet immunoglobulins, was not affected by SCFP in the diet of sows. Hasan et al. (2018) showed that gestating sows fed yeast derivatives had greater colostrum yield and fat content while not affecting the immunoglobulins levels. In our study, the lack of differences in colostrum Brix values, a good indicator of colostrum IgG concentration (Balzani et al., 2016; Hasan et al. 2016), resulted in no difference in plasma IgG and IgA concentrations of pigs 24 h after birth as well as immunocrit ratio. These results agree with a previous study showing that SCFP fed to gestating and lactating sows did not affect IgG concentration in colostrum nor pig`s blood while improved maternal health status, as shown by a reduction in neutrophil cell count (Shen et al., 2011). On the other hand, feeding a specific strain of *Saccharomyces cerevisiae* to sows, during gestation and lactation period, had improved colostrum IgG and milk IgA concentrations, enhancing litter health status as diarrhea incidence reduced (Zanello et al., 2013). These differences among studies may be explained by inherent differences among feed additives, the duration of feeding the additive, the herd immunological status, feeding programs, and nutritional levels of the diets. Nevertheless, SCFP are expected to have immunomodulatory effects since they are rich in compounds prebiotics like such as mannan-oligosaccharides, β-glucans, and other yeast fermentation metabolites and derivates (Price et al., 2010).

As expected, the litter size was not affected by dietary treatments since SCFP were given only approximately three days before sows farrowed. This study has not shown differences in pre-weaning mortality but litter uniformity at weaning was improved by SCFP fed to sows, as shown by the lower percentage of pigs lighter than 3.5 kg and the greater improvement of the BW coefficient of variation from birth to weaning. Reduction in light BW pigs and variability at weaning is an important productive parameter to take into account when evaluating any strategies to improve lactating sows performance as it is related to efficiency and occupation time of nursery and grow-finishing facilities as well as to body weight of pigs at slaughter.

With the increased sow feed intake and greater litter weight gains of sows fed the SCFP we expected to potentially see water intake to increase. However, in this study, no effects of SCFP were observed in daily water intake. Kruse et al. (2011) showed that lactating sows drink water 4.7 to 5.2 times the amount they eat, which represents approximately 26 to 32 L/day. Present results are consistent with this range at an average of 5.4 times.

The subsequent reproductive performance in sows, mainly WEI, is a good indicator of the degree of body reserve mobilization and energy balance during the previous lactation. Sows fed LIQ had a reduction of 0.5 day on WEI compared to CON which could be explained by the greater feed intake and lower BW change observed in this group of sows. Other studies found different results on subsequent reproductive performance feeding SCFP to sows. Kim et al. (2010) observed a reduction on WEI while Shen et al. (2011) did not find improvements on WEI. The inconsistent results regarding subsequent reproductive performance might be related to body condition of the sows at farrowing and the extent of mobilization during lactation.

In summary, SCFP fed to lactating sows improved feed intake and litter growth while not affecting colostrum quality and immunoglobulins in the offspring. Pig weight at weaning was improved from sows given the liquid source of SCFP while pig ADG was improved regardless the source of SCFP. Sows with the greatest feed intake had less body weight mobilization and a reduction on WEI. In conclusion, SCFP improved sows lactation and litter performance possibly through promoting a quicker recovery after farrowing. Further investigations regarding the mode of action of SCFP around parturition and improved lactation feed intake are needed.

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The Impact of Nutrition on Barrier Function in the Young Pig

Sheila K Jacobi, Rui Zheng, and Emily Chucta
Department of Animal Sciences
The Ohio State University, Columbus, OH 43210
Phone: 614-247-7863
jacobi.1@osu.edu

Summary

Optimal pig performance is fundamental to the profitability of the swine industry and high performing pigs are highly correlated with optimum intestinal health. The suckling and post-weaning piglets are among the most susceptible animals to diarrheal disease associated with opportunistic pathogens which leads to periods of high morbidity and mortality (USDA, 2012). The animal livestock industry has utilized antimicrobial growth promotants to mitigate some of the challenges of intestinal health and to maximize growth efficiency. However, the need to reduce the feeding of antimicrobial growth promotants in livestock production has driven the industry to look at alternative ways to optimize growth performance while minimizing use of antimicrobials. Nutrition has a great impact on intestinal health and understanding nutritional mechanisms that promote intestinal health and barrier function could have an impact on production efficiency. Likely, optimizing intestinal health is going to require a multiple pronged approach including utilizing nutritional supplementation to target improving intestinal health and intestinal barrier function, along with, cultivating optimal on-farm management practices that allow application of target feed supplements. The development of different management and feeding strategies to enhance intestinal health means understanding mechanistically how nutrients are affecting the intestinal barrier under healthy and challenged environments. Nutritional regulation of the intestinal barrier function in early life could have lifelong impacts on the intestinal barrier and immune function throughout the production cycle. Therefore, we investigated the mechanistic role of long-chain polyunsaturated fatty acids on mechanisms of intestinal barrier function.

Introduction

Gastrointestinal health issues rank among the highest causes of neonatal morbidity and mortality across most mammalian species, including domestic livestock. Prior to weaning, gastrointestinal distress in the form of scours accounts for 10.2% of pre-weaning piglet mortality, with 47.8% of herds having *E. coli* disease problems in pre-weaned piglets (USDA, 2012). Enterotoxigenic *E. coli* (**ETEC**) is the most common type of colibacillosis of young animals and is overall the second most prevalent disease in young pigs (Nagy and Fekete, 2005). At the onset of intestinal infections, the host inflammatory responses are initiated, and intestinal morphology and function are compromised. Inflammation and cytokines have a marked effect on intestinal barrier permeability (Dubreuil et al., 2016). Using nutrition preventatively instead of reactively requires the understanding of how nutrients and their metabolites are affecting the intestinal luminal environment, intestinal barrier and the underlying mucosal immune system.

Multiple dietary supplements are being investigated to optimize swine intestinal health and barrier function to optimize production efficiency. Long-chain polyunsaturated fatty acids (**LC-PUFA**): arachidonic acid (**ARA**), eicosapentaenoic acid (**EPA**), and docosahexaenoic acid (**DHA**) and their lipid metabolites have been shown both to alter intestinal permeability and the inflammatory state of the intestine. Intestinal permeability has been shown to be enhanced through increases in transepithelial electrical resistance (**TEER**), decreases in large molecule flux, and increases in expression of tight junction (**TJ**) proteins critical in maintaining the passage through the paracellular pathway (Suzuki, 2013). LC-PUFA and the lipid metabolites derived from them have been characterized as ligands for regulating gene transcription through specific protein mechanisms such as peroxisome proliferator activated receptor gamma (PPARγ) (Martin, 2010). PPARγ has been characterized as being anti-inflammatory through its ability to inhibit activation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated

B cells (NF-κB) and reduced expression of inflammatory genes, such as cytokines (Martin, 2010, Zehmon et al., 2011). Patterson et al. (2008) fed sows 2% linoleic acid (C18:2, n-6), the ARA precursor, and then challenged the piglets with ETEC K88+ and found less severe scours and reduced intestinal mucosal inflammation compared to piglets weaned from control fed sows (Patterson et al., 2008). The omega-3 fatty acids, DHA and EPA, additionally have been shown to act as antimicrobials and reduce viability of *E. coli*, with DHA having more potent effects than EPA (Chanda et al., 2018; Casillas-Vargas et al., 2021). Despite therapeutic potential, little research has been completed to determine if LC-PUFA could have protective effects on intestinal barrier function during an *E. coli* infection in neonates. To understand the modulatory mechanisms of LC-PUFA on barrier function and inflammation the intestinal neonatal cell line, IPEC-J2 cells, were challenged with ETEC K88+ in the presence or absence of LC-PUFA.

Methods

Cell culture. The intestinal porcine epithelial jejunal (IPEC-J2) cell line was a kind gift from Dr. Kolapo Ajuwon of Purdue University. The IPEC-J2 cells used in this study are non-transformed, polarized- porcine jejunal epithelial cells and were originally isolated from a neonatal, unsuckled piglet (Vergauwen, 2015). Cells were cultured at 37°C at 5% CO_2 in DMEM/F12 media (Sigma-Aldrich, Saint Louis, MO) with 5% fetal bovine serum (Atlanta Biologicals - Flowery Branch, GA), 5 μg/mL Insulin, Transferrin and Selenium (Corning, Sigma-Aldrich, Saint Louis, MO), 5 μg/mL Epidermal Growth Factor (Corning, Sigma-Aldrich- Saint Louis, MO) and Penicillin Streptomycin (Gibco, Gaithersburg, MD) until challenge.

Fatty acid treatments. IPEC-J2 were seeded and grown in 12-well plates (Corning Costar® Transwell® Permeable supports, Corning, Sigma-Aldrich- Saint Louis, MO). Once cells were confluent on day 9 LC-PUFA treatments were applied. Control of Bovine Serum Albumin (BSA) and fatty acids complexed to BSA: Oleate (OA; C18:1), ARA (C20:4), EPA (C20:5) and DHA (C22:6) were added into individual cell wells in media at a concentration of 30 μM to allow for enrichment of cellular membranes. The day before addition of bacteria the media was changed to contain heat inactivated FBS and no antibiotics.

Bacteria for challenge assay. Enterotoxigenic Escherichia coli (**ETEC**) strain 3030-2: K88ac, LT, STb was kindly provided by Dr. Philip Hardwidge of Kansas State University. The strain was grown at 37°C overnight and then diluted 1:10 in Luria Broth, grown for 90 minutes and then bacterial population were estimated by spectrophotometry at 600nm optical density. ETEC was added to the media in the apical compartment at the multiplicity of infection (MOI) of 2:1 for 6 hours.

Trans-epithelial electrical resistance. Epithelial cell barrier integrity was assessed through measurement of trans-epithelial electrical resistance (TEER) at hour 0, 2, 4, and 6 of ETEC incubation. TEER measurements were conducted using an EVOM2 epithelial voltohmmeter (World Precision Instruments, Sarasota FL). Individual wells were measured in triplicate and average values were expressed as Ohms x cm^2 ($\Omega \cdot cm^2$).

Paracellular flux determination. After 6 hours of ETEC incubation, cells were washed three times with 1X PBS. Paracellular permeability was measured using 4-kDa fluorescein isothiocyanate-dextran (FITC-dextran; Sigma-Aldrich, Saint Louis MO). FITC-dextran was added at a concentration of 1 mg/mL to the apical insert and cultured at 37°C for 1 hour. Medium from each basolateral compartment was collected and fluorescence intensity measured at an excitation wavelength of 480 nm and an emission wavelength of 520nm.

Real time PCR analysis. Cells were washed in PBS and collected for RNA isolation using RNAzol® RT (Molecular Research Center Inc., Cincinnati, OH). One μg of RNA was used to conduct reverse transcription cDNA synthesis utilizing the iScript cDNA synthesis kit (Bio-Rad- Hercules, CA). Primers were designed for this study using NCBI primer blast and amplification of correct gene product was evaluated by gel electrophoresis and efficiency was evaluated. Amplicons were sequenced. Quantitative real time PCR was performed using Applied Biosystems® 7500 real-time PCR system (ThermoFisher Scientific, Waltham MA) using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, Hercules CA). Controls of no template and no reverse transcriptase samples were utilized and melt peaks were analyzed to ensure there was no contamination or genomic DNA interference, respectively. mRNA abundance of genes of interest and internal control gene for each sample were ran in triplicate and ΔΔCT was used for analysis. Ribosomal protein L4 (RPL4) was used as the internal control gene.

ELISA. Media collected in experiments from the basolateral compartment after 6 hours ± ETEC challenge was frozen at -80°C until time of analysis. Commercially available swine enzyme-linked immunosorbent assay kits for cytokines and chemokines IL-6 and IL-8 (Invitrogen by Thermo Fisher Scientific, Waltham MA) were used according to manufacturer's protocols.

Immunohistochemistry of NF-kB. Cells that were grown in 12-well plates were utilized for immunohistochemistry (**IHC**). After 6-hour challenge, cells were washed in 1x PBS and fixed with 4% formaldehyde. For cells analyzed for nuclear NF-κB primary antibody was

p-NF-kB P65 (Cell Signaling Technologies 93H1, Danvars, MA) used at 1:500 and for nuclear PPARγ primary antibody was (Invitrogen REF#PA3-821A, Waltham, MA) used at 1:2000. Secondary antibody was Alexa Fluor® 488 (Invitrogen, Carlsbad, CA) and nuclei were stained with DAPI (ThermoFisher Scientific, Waltham MA). Images were taken using EVOS FLc (Invitrogen, Carlsbad, CA) and intensity of p-NF-kB-P65 or PPARγ within the nucleus was quantitatively analyzed using the ImageJ software.

Statistical analysis. All data were analyzed as a 2 X 5 factorial design using the PROC MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The TEER data additionally included repeated measures in time with various covariate structures of error fitted; the toeplitz error structure was selected based on the lowest Bayesian Information Criterion (**BIC**). The model included the fixed effects of fatty acid treatment (**FA**), ETEC treatment (**ETEC**), their interaction (FAxETEC) and the random effect of well. Treatment differences were compared using the DIFF option of the LSMEANS statement with employs a Bonferonni's protected least significance difference (**LSD**) test. The α-level for significance was set at $P < 0.05$. Data were reported as means ± SEM for a given number (n) of wells for each experiment.

Results

Fatty Acid Enrichment of Cell Membrane. When ARA, EPA, or DHA were supplemented into the media for 96 hours it resulted in 5.7-fold, 41-fold, and 21-fold increases in fatty acid composition within IPEC-J2 phospholipid membranes compared to control IPEC-J2 cell membranes, respectively ($P < 0.05$; Table 1).

Barrier permeability. To determine how LC-PUFA treatment and ETEC challenge effected paracellular barrier integrity, we first measured TEER at the beginning of the challenge as well as at hours 2, 4, and 6. There was a significant reduction in TEER by 4 and 6 hours compared to time zero reading caused by ETEC challenge and regardless of fatty acid treatment ($P < 0.001$; Figure1A). Paracellular flux of FITC-dextran was analyzed at 6 hours post-challenge. Compared to BSA control and ARA treatment, EPA and DHA treatments increased FITC-dextran flux through the membrane by 2.15-fold ($P = 0.04$) and 2.27-fold ($P = 0.01$), respectively (Figure 1B).

Table 1. Membrane LC-PUFA composition following supplementation with 30 µM LC-PUFA after 96 hours in IPEC-J2 cells[1].

		30 µM LC-PUFA supplementation for 96 hours						
		CON	**OA**	**ARA**	**EPA**	**DHA**	**SEM**	**P-value**
% of LC-PUFA in phospholipid membrane	**OA**	31.65^{ab}	32.26^{a}	29.56^{c}	30.11^{bc}	32.48^{a}	0.89	P < 0.05
	ARA	0.96^{b}	0.70^{b}	5.47^{a}	1.02^{b}	0.98^{b}	0.39	P < 0.05
	EPA	0.03^{b}	0.18^{b}	0.16^{b}	1.23^{a}	0.23^{b}	0.13	P < 0.05
	DHA	0.02^{d}	0.23^{c}	0.26^{c}	0.87^{a}	0.42^{b}	0.11	P < 0.05

[1] Values are means ± SEM, n = 6. Different letters differ significantly, P < 0.05.
CON, control BSA; OA, oleic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Tight junction protein mRNA expression. Due to alterations of flux of molecules through the barrier by LC-PUFA, mRNA abundance of tight junction proteins were measured because the proteins are critical in the maintenance of the paracellular pathway. ETEC challenged cells compared to unchallenged cells had reduced occludin mRNA abundance by 1.5-fold ($P < 0.01$; Figure 2A) and ZO-1 mRNA expression was reduced by 1.7-fold ($P < 0.001$; Figure 2B). Occludin mRNA expression of EPA treated cells was reduced by 2.12-fold relative to DHA treated cells ($P < 0.01$) and reduced by 1.72-fold compared to OA treated cells ($P = 0.04$; Figure 2A). ZO-1 mRNA expression of EPA treated cells was reduced 1.58-fold relative to ARA treated cells ($P = 0.04$), reduced 1.68-fold relative to BSA control ($P = 0.02$) and reduced 1.81-fold relative to DHA treated cells ($P < 0.01$; Figure 2B).

LC-PUFA alteration of cytokine response. Cytokines can alter the membrane permeability of intestinal cells. The effect of LC-PUFA treatment and ETEC challenge on cytokine mRNA abundance and protein excretion of IL-6 and IL-8 were measured. Cytokine mRNA abundance and protein secretion in IPEC-J2 cells was regulated by FA and challenge. IL-6 mRNA abundance was differentially regulated by FA treatment ($P = 0.03$). Oleate and DHA treated cells had increased IL-6 mRNA abundance by 1.86-fold ($P = 0.002$) and 1.68-fold ($P = 0.01$) relative to ARA treated cells, respectively (Figure 3A). Oleate treated cells additionally had increased IL-6 mRNA abundance by 1.5-fold relative to EPA treated cells ($P = 0.02$; Figure 3A). Protein secretion of IL-6 increased 3.46-fold in ETEC challenged cells relative to unchallenged cells ($P < 0.001$; Figure 3C). Within the unchallenged cells treatments, Oleate and DHA treated cells had a 4-fold increase in IL-6 secretion relative to ARA and EPA ($P < 0.05$; Figure 3C). IL-6 protein secretion in all FA treatments in challenged cells, except for DHA, had a significant IL-6 increase compared to the FA treatments that were not challenged ($P < 0.05$; Figure 3C). The BSA(CON), OA, ARA, and EPA treated cells in ETEC challenged groups had 3.19-fold ($P = 0.001$), 1.99-fold ($P = 0.02$), 8.4-fold ($P < 0.001$), and 9.06-fold ($P < 0.001$) increase in IL-6 protein secretion relative to unchallenged cells (Figure 3C).

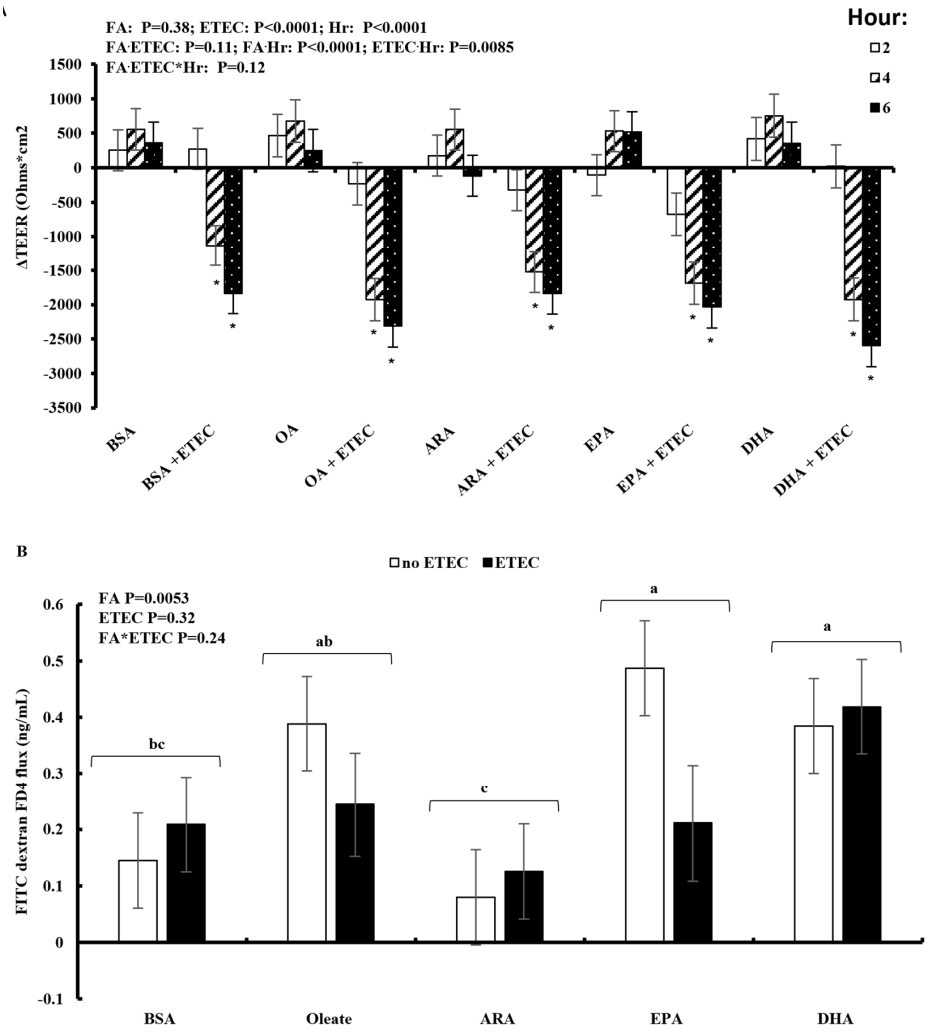

Figure 1 Intestinal barrier permeability of IPEC-J2 cells treated with LC-PUFA ± ETEC challenge at a MOI of 2:1 on the apical membrane for 6 hours as measured by TEER (A) and FITC-dextran flux (B). TEER bars represent treatment mean change of TEER at hour 2, 4, and 6 post-challenge compared to hour 0 mean ± SEM, n = 6. * represents difference in TEER at P < 0.05 compared to treatment TEER at hour 0. FITC-dextran flux is reported from the apical to the basolateral side of IPEC-J2 cells at 6 hour post-ETEC challenge. Bars represent treatment means ± SEM, n = 6. Different letters represent fatty acid effects that differ at P < 0.05.

For IL-8 measurements differences in the main effect of FA, ETEC and interactions were seen for mRNA abundance and a main effect of FA was seen for protein secretion. IL-8 mRNA abundance was increased by 8.17-fold in ETEC challenged cells compared to unchallenged cells ($P < 0.001$; Figure3B). Additionally, IL-8 mRNA abundance was differentially regulated by fatty acid treatments with ETEC challenge (Figure 3B). IL-8 mRNA abundance in EPA treated + ETEC cells was 2.13-fold ($P = 0.001$) and 1.79-fold ($P = 0.005$) higher than BSA(CON) treated + ETEC and ARA treated + ETEC cells, respectively (Figure 3B). DHA treated + ETEC cells had greater IL-8 mRNA abundance 4.08-fold, 3.42-fold, and 1.91-fold higher than BSA (CON), ARA, and EPA treated + ETEC cells, respectively ($P < 0.0001$; Figure 3B). IL8 protein secretion also was altered by FA treatments ($P = 0.01$; Figure 3D). DHA treated cells had 2-fold increase in IL-8 secretion compared to BSA(CON) and ARA treated cells ($P < 0.01$; Figure 3D). Additionally, OA and EPA treated cells had 2-fold increase in IL-8 protein secretion compared to BSA(CON) treated cells ($P < 0.05$; Figure 3D).

LC-PUFA alteration of IκBα mRNA expression and NF-κB nuclear abundance. Because there were observed alterations of cytokines the transcription factor, NFκB, which is a major regulator of pro-inflammatory pathways was measured. Inhibitor of NF-κB α (IκBα) is responsible for inhibiting NF-κB nuclear localization and function is critical for increasing the immune response. At 6 hours post ETEC challenge there was an average increased IκBα mRNA abundance of 2.3-fold compared control cells, except for in EPA challenged cells ($P <$

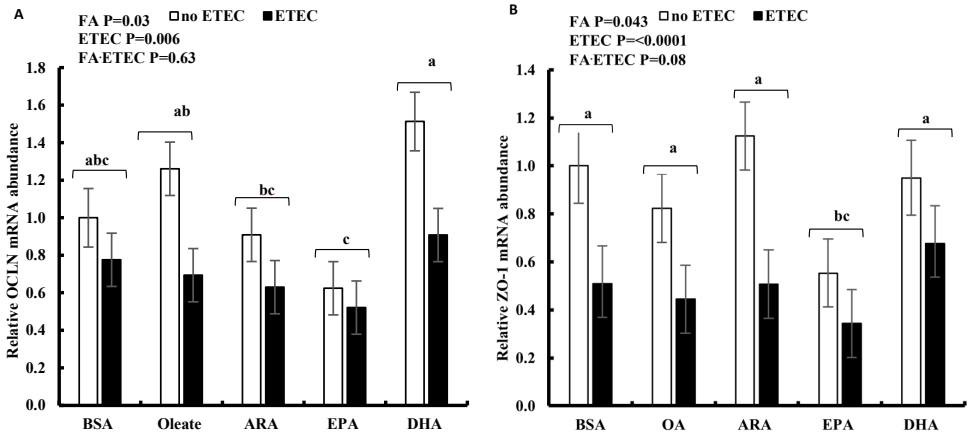

Figure 2. Tight junction protein occludin (A) and ZO-1 (B) mRNA abundance in IPEC-J2 cells treated with LC-PUFA ± ETEC challenge on the apical membrane for 6 hours. Bars represent treatment means relative to control ± SEM, n = 6. Different letters represent effects that differ at $P < 0.05$.

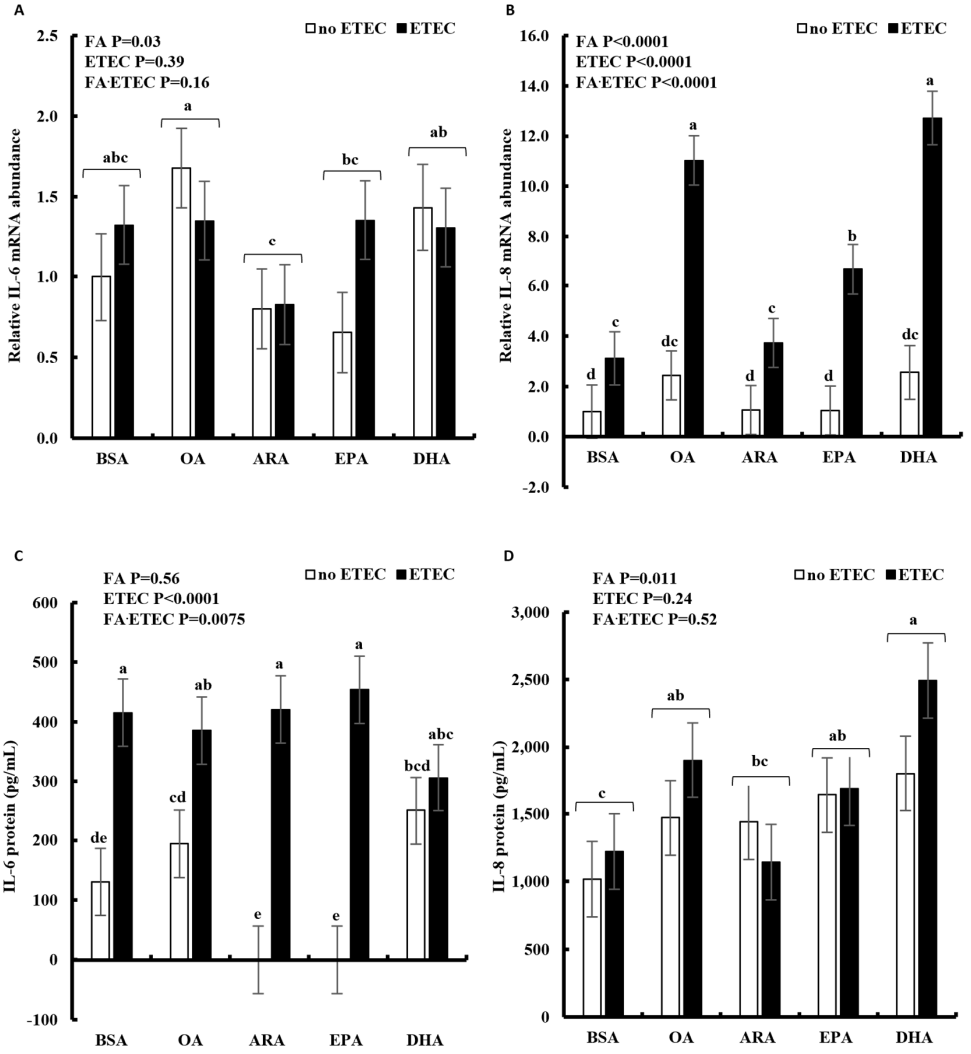

Figure 3. Cytokine response of IL-6 (A) and IL-8 (B) mRNA abundance in IPEC-J2 cells and IL-6 (C) and IL-8 (D) protein secretion in IPEC-J2 cells pre-treated with LC-PUFA ± ETEC challenge for 6 hours. mRNA expression (A and B) bars represent treatment means relative to control ± SEM. Protein secretion bars represent treatment means ± SEM. Different letters represent fatty acid effects that differ at $P < 0.05$ (n = 6).

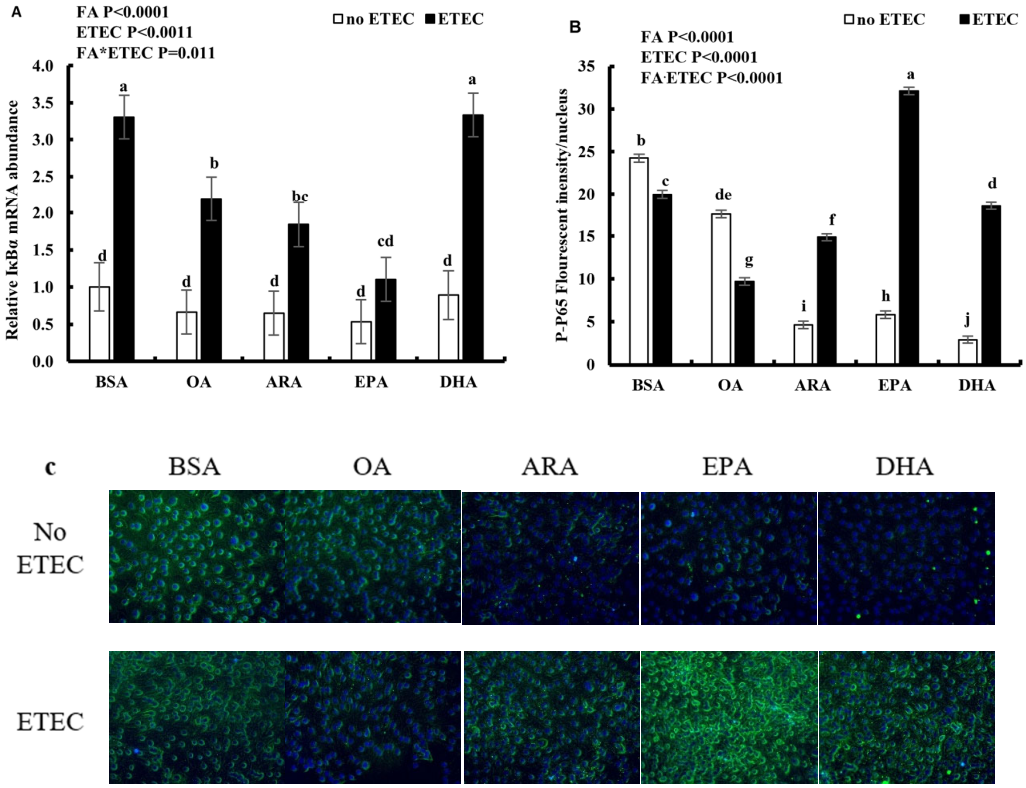

Figure 4 Measures of cellular NFkB signaling in IPEC-J2 cells treated with LC-PUFA ± ETEC challenge. NF-kB1A(IκBα) mRNA abundance (A); quantification NFkB p-P65 nuclear fluorescent intensity (B); 20x objective confocal microscopy of NFkB p-P65 nuclear localilization (C). IκBα mRNA expression (A) bars represent treatment means relative to control ± SEM. NFkB p-P65 nuclear fluorescent intensity (B) bars represent average intensity per nucleus ± SEM. NFkB p-P65 nuclear localization (C) represents pictures that were analyzed for quantitative nuclear florescent intensity analyzed in panel B; blue stain is DAPI for staining the nucleus and green stain stains NFkB p-P65. Different letters represent differences at P < 0.05, n = 6.

0.001;Figure 4A). Fatty acids differentially modulated the mRNA abundance of IκBα in ETEC challenged cells. Oleate, ARA, and EPA treated cells + ETEC had decreased mRNA abundance of IκBα compared to challenged BSA control or DHA treated cells (Figure 4A). Challenged EPA treated cells had the lowest IκBα mRNA abundance of all challenged cell treatments and was not significantly different from EPA unchallenged cells (Figure 4A).

The fluorescent intensity of p-NF-kB-P65 localization to the nucleus was determined by immunohistochemistry. ETEC challenged cells had increased nuclear intensity of p-NF-kB P65 by 1.7-fold compared to unchallenged cells ($P < 0.001$; Figure 4B). Compared to unchallenged BSA control, FA treatments without challenge significantly reduced p-NF-kB P65 nuclear intensity with a reduction in intensity by 1.43-fold (OA), 5.36-fold (ARA), 4.07-fold (EPA) and 8.43-fold (DHA) relative to CON ($P < 0.001$; Figure 4B). Compared to challenged BSA control, FA treatments differentially regulated nuclear intensity of p-NF-kB P65 in the nucleus (Figure 4B; $P < 0.001$). Challenged cells treated with OA, ARA, and DHA had 2.05-fold, 1.34-fold, and 1.08-fold reduction in nuclear intensity (Figure 4B and 4C), whereas challenged cells with treatment of EPA had 1.61-fold increase in p-NF-kB P65 nuclear intensity compared to challenged BSA (Figure 4B). All LC-PUFA also differentially modulated intensity compared to other LC-PUFA treatment when challenged (Figure 4B; $P < 0.001$). When challenged, EPA treated cells had increased intensity by 2.25-fold and 1.76-fold relative to ARA and DHA treated cells, respectively (Figure 4B and 4C). DHA treated cells when challenged also increased intensity by 1.25-fold compared to ARA treated cells (Figure 4B and 4C).

LC-PUFA regulation of PPARγ pathway. To determine alterations of PPARγ activity we analyzed PPARγ mRNA abundance and fluorescent intensity in unchallenged and challenged IPEC-J2 cells. Fatty acid treatment decreased PPARγ mRNA abundance by an average of 1.67-fold compared to BSA control regardless of ETEC challenge ($P < 0.001$; Figure 5A). Nuclear fluorescent intensity of PPARγ was not significantly altered by any treatment or ETEC challenge ($P > 0.05$; Figure 5B).

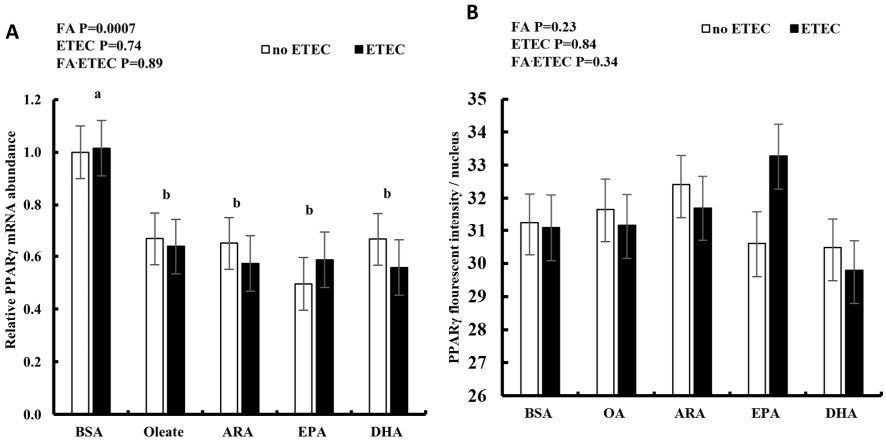

Figure 5. mRNA expression of PPARγ (A) and analysis of PPARγ fluorescent intensity/ nucleus (B) in IPEC-J2 cells treated with LC-PUFA ± ETEC. mRNA abundance of PPARγ (A) bars represent means relative to control ± SEM, n = 6. PPARγ nuclear fluorescent intensity (B) bars represent average intensity per nucleus ± SEM. Different letters represent effects at $P < 0.05$.

Conclusions

The high prevalence worldwide of enteric diseases and disfunction in domestic livestock has led to much interest in understanding the role of dietary nutrients in the establishment and maintenance of optimal functioning gastrointestinal tract. Intestinal maladies such as ETEC infection cause intestinal disfunction of the gate-keeping intestinal epithelial cells that alter intestinal permeability and inflammation. The objective of this study was to understand the mechanisms of polyunsaturated fatty acids on intestinal barrier function using the *in vitro* IPEC-J2 cells model in unchallenged and ETEC challenged cells.

There are two common measures of intestinal barrier function, the resistance across the epithelial cell layer (poor pathway) and paracellular passage of molecules from the apical (lumen) to the basolateral (lamina propria) side of the epithelial cell layer (leak pathway) (Shen et al., 2011). Tight junctions are important structures in regulation of paracellular molecule flux in the intestine. Occludin and ZO-1 are important regulators of the leak paracellular pathway that allows macromolecule flux through the barrier (Shen et al., 2011). In our study, IPEC-J2 pig intestinal cells challenged with ETEC at an MOI of 2:1 had a reduction in TEER at 4- and 6-hours post challenge regardless of LC-PUFA treatment. Although others have shown LC-PUFA have protective effects on intestinal barrier function TEER under inflammatory cytokine challenge (Willemsen et al., 2008; Li et al., 2008), our study used an isolated strain of *E. coli* from nursery pigs known to cause diarrheal disease. The live bacterial ETEC strain used in this study has been characterized to secrete heat labile toxin (Johnson et al., 2010) which can decrease TEER through a cystic fibrosis transmembrane conductance regulator (CFTR)-dependent mechanism regulating Cl^- transport and diarrheal disease (Fleckensteina et al., 2010). In the IPEC-J2 + ETEC model we used there are multiple mechanisms by which the live pathogen could disrupt intestinal barrier function not only an inflammatory stimuli of cytokines and activating ion channels may be a mechanism not regulated by LC-PUFA in an acute challenge. However, the paracellular flux of FITC-dextran (pore pathway) was modulated by supplemental fatty acids regardless of ETEC challenge. Compared to control and ARA supplemented cells the fatty acids oleic acid, EPA and DHA had increased molecule flux from the apical to basolateral side of the epithelial barrier (Figure 1B), and in the case of EPA was associated with a reduction in the tight junction proteins, occludin and ZO-1 mRNA abundance. The measure of mRNA transcripts for the tight junction proteins only provide gene level mechanisms so measures of tight junction protein abundance and localization will provide important mechanisms of associated molecule flux and regulation by LC-PUFA supplementation.

LC-PUFA n-3 LC-PUFA (EPA and DHA) and n-6 LC-PUFA (ARA) treatments differentially modulate the inflammatory response through regulation of proteins involved in inflammatory cytokine production. IκBα mRNA and NF-κB protein expression are responsible for turning on and off inflammatory signals in tissues. These data showed that in unchallenged and ETEC challenged IPEC-J2 cells that LC-PUFA or lipid metabolites altered the inhibitor of NFkB and NFkB nuclear location in cells. The NFkB transcription factor and associated cytokines are responsible for regulation (on set and resolution) on the inflammatory and immune response. The molecules associated with the regulation of the onset and resolution of inflammation are com-

plex but are altered by LC-PUFA. Based on our data n-3 LC-PUFA seem to increase barrier permeability and inflammation acutely under ETEC challenge compared to n-6 LC-PUFA. This is in contradiction to other inflammatory challenge models in pigs where Lui et al. (2012) showed n-3 LC-PUFA may have an anti-inflammatory role in intestinal barrier function permeability when weaning pigs were challenged with systemic lipopolysaccharide. Many factors likely are involved in these differences, in particular the difference between a systemic inflammatory molecule such as lipopolysaccharide and an enteric pathogen, like ETEC. Mechanistically these challenge models, while they may have similarities, also have unique mechanisms of disruption of intestinal barrier integrity. Furthermore, our *in vitro* system does not benefit from the animal local and systemic immune function but serves as a tool to investigate some biochemical mechanisms that could be important in the onset and resolution of inflammation in pig intestinal epithelial cells. Our data indicates there are roles of LC-PUFA in modulating transcriptional inflammatory mechanisms following acute ETEC challenge. However, a further understanding of how the biochemical pathways and mechanisms are altered under different doses and time challenges could provide greater understanding of how the nutrient could be applied to improve intestinal health and production performance.

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The Effect of Medium Chain Fatty Acids on Porcine Reproductive and Respiratory Syndrome Virus

S. A. Crowder, R. Pogranichniy, and J. S Radcliffe
Department of Animal Sciences
Purdue University
West Lafayette, IN
Phone: 765-496-7718
jradclif@purdue.edu

Summary

Porcine Reproductive Respiratory Syndrome Virus (PRRSV) costs the U.S. swine industry greater than $600 million in production losses annually. Medium chain fatty acids (MCFA) have emerged as a feed additive that enhance animal health and are beneficial as a viral feed mitigant. Therefore, a series of experiments were conducted to investigate the effects of individual and combinations of medium chain fatty acids (MCFAs) on PRRSV replication in a PRRS challenge cell culture model. Individual MCFAs of 8, 10 or 12 carbons or combinations of MCFAs were able to reduce PRRS viral replication. In general, as MCFA chain length increased (from 8 to 12), the amount of MCFA needed to reduce viral replication decreased.

Introduction

The swine industry has been plagued by Porcine Reproductive Respiratory Syndrome Virus (PRRSV) for over 30 years (Brar et al., 2014). The virus was first isolated in 1991 in Europe and 1992 in the US leading to the identification of 2 PRRSV genotypes: the type I European and type II North American. PRRSV is an enveloped, positive, single-strand RNA virus. The virus can be transmitted vertically (sow to piglet) and horizontally (pig to pig) by nasopharyngeal and aerosol routes. PRRSV infection results in reproductive failure in sows and gilts as well as respiratory illness in all phases of pig production. Reproductive failure in sows can range from decreased farrowing rates to increased abortions and low viability in liveborn pigs. Respiratory disease severity increases with the presence of secondary infections leading to high mortality rates in pre- and post-weaned pigs. It has been estimated that PRRSV infection costs the US Pork Industry $664 million annually in production losses (Holtkamp et al., 2013). Approximately 55% of the economic losses are due to high mortality, decreased growth rates, and respiratory distress in growing pigs.

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

This has brought about increased interest in research focused on nutritional implications or strategies to improve animal health. Nutritional impacts on health require communication between the gastrointestinal tract, the central nervous system, and the immune system (Rehfeld, 2004; Janssen & Depoortere, 2013). Communication between the gut and other organ immune

functions such as Gut-Lung, Gut-Brain, Gut-Liver provide evidence to support the influence of nutrition on immune responses (Xiao et al., 2011; Furness et al., 2013; Budden et al., 2017; Ge et al., 2021)it must be accessible and permeable to nutrients and must defend against pathogens and potentially injurious chemicals. Integrated responses to these challenges require the gut to sense its environment, which it does through a range of detection systems for specific chemical entities, pathogenic organisms and their products (including toxins.

Medium chain fatty acids (**MCFA**) have emerged as a target feed additive to improve animal health and performance (Cochrane et al., 2016; Gebhardt et al., 2020). MCFA can shift microbial populations by inhibiting pathogenic bacteria and thereby allowing an increased growth of beneficial bacteria (Yoon et al., 2018). This knowledge provides the foundation that nutrition can impact animal health and performance. Research on intestinal morphology and gut microbiota have reported an increase in intestinal villi height and crypt depth as well as modified microbiota with the use of MCFA in pig diets (Dierick et al., 2003; Calder, 2008; Zentek et al., 2012). Understanding the vast interaction of the gut with other regulatory systems is still not complete but does provide new targets for impacting health and performance in the animal as well as new targets for therapeutic products to prevent infection and disease spread.

Viral transmission through feed has been of increased interest following the emergence of Porcine Epidemic Diarrhea Virus (**PEDV**) in 2013. Research on feed mitigation has reported that MCFA are effective at reducing viral particles and infectivity of feed and feed ingredients (Lerner et al., 2020). These findings suggest that it is possible to influence animal health and the immune response through nutrition. Therefore, the objective of these experiments was to investigate the potential of MCFAs to decrease viral replication in a PRRS challenge cell culture model.

MCFAs in Cell Culture

A series of experiments were conducted to investigate the impact of individual or combinations of MCFAs on an in vitro PRRSV infection model. Meat Animal Research Center -145 (MARC-145) cells, which are a subclone of African Green Monkey Kidney MA104 cells, and are highly permissive to PRRSV, were used as an immortal cell model.

Briefly, MARC-145 cells were seeded onto 96 well tissue culture plates and incubated at 37°C in a 5% CO_2 environment for 48 h. After the 48 h incubation, wells were washed with growth media and exposed to MCFA concentrations of 0-1,000 mg/mL. Plates were then incubated for 2 h, washed with growth media and inoculated with 100 µL of a 50:50 mixture of virus and MCFA or growth media. Plates were then incubated for 1 h, and then virus was removed, and plates were washed with growth media. MCFA concentrations were added to each well at a volume of 100 µL/well, and plates were incubated for 48 h at 37°C in a 5% CO_2 environment.

MARC-145 cell plates were removed from the incubator after 48 h and wells were washed with phosphate buffered saline, fixed with acetone, blocked with bovine serum albumin blocking buffer and stained with FITC labeled IgG anti-PRRSV monoclonal antibody (SDOW17-F RTI Labs Brookings, South Dakota) to allow visualization under a UV microscope to determine viral replication within the MARC-145 cells. The median tissue culture infective dose Log_{10} $TCID_{50}$/mL was calculated for each MCFA concentration and control (Karber, 1931).

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

Results

Incubation of MARC-145 cells with caproic acid (C6) at concentrations of 0-1000 µg/mL prior to and after inoculation with Type II North American (P129) or Type I European (Lelystad) PRRSV did not alter viral replication ($P > 0.10$). However, incubation of MARC-145 cells with caprylic (C8), capric (C10), and lauric (C12) acid prior to and after inoculation with Type II and Type I PRRSV did lead to reductions in viral replication. In general, the effective dose required to result in a reduction in Log_{10} $TCID_{50}$/mL ($P < 0.05$) viral replication decreased as MCFA chain length increased (Figure 1).

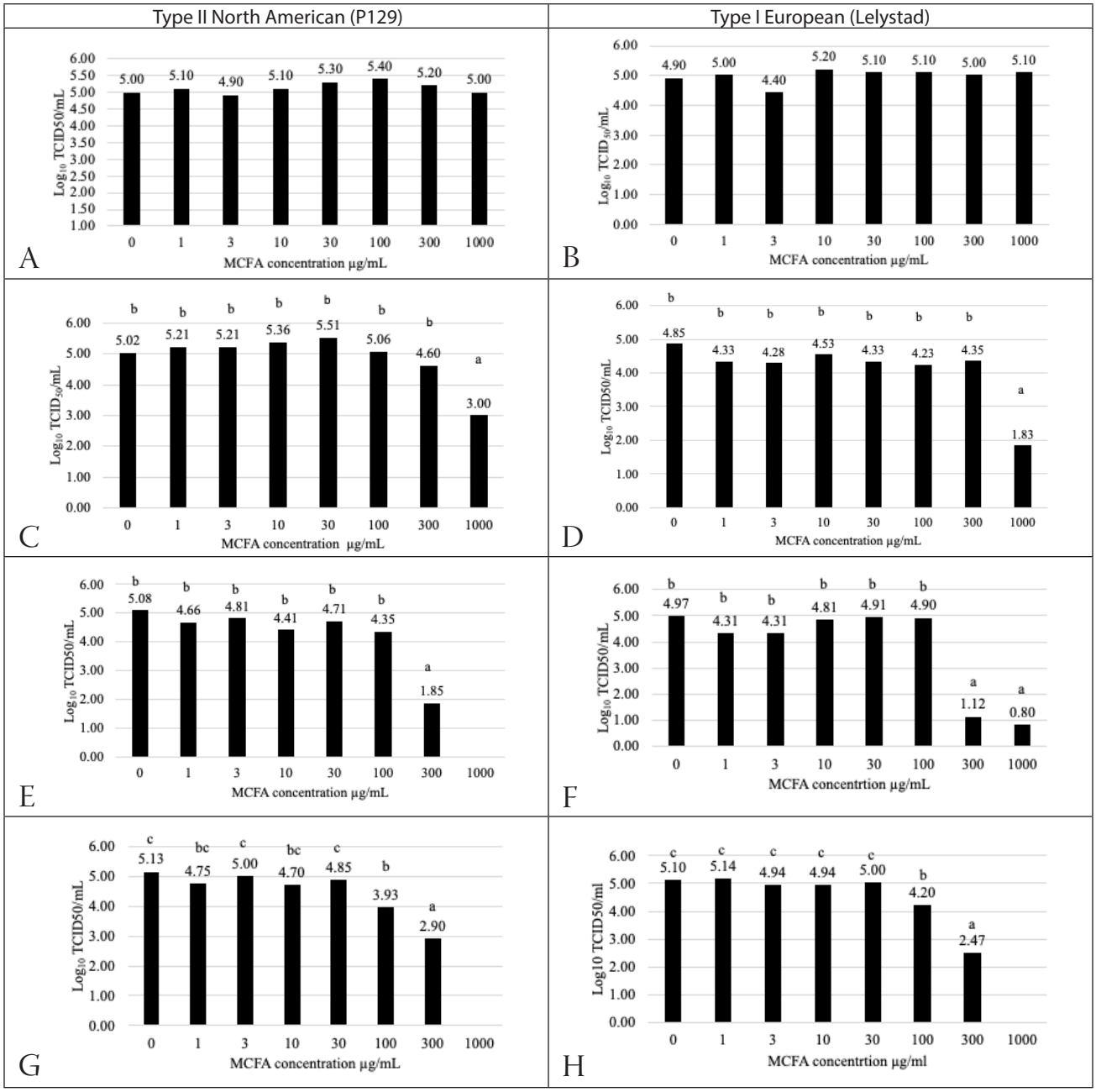

Figure 1. The effects of incubating M145 Cells with caproic (C6; panels A & B), caprylic (C8; panels C & D), capric (C10, panels E & F), or lauric (C12, panels G & H) acid prior to and after inoculation with Type II North American (P129) or Type I European (Lelystad) PRRSV on viral replication. [abc]Letters indicate differences ($P < 0.05$).

Incubation of MARC-145 cells and virus with MCFA combinations prior to and after inoculation with Type II North American (P129) and Type I European (Lelystad) PRRSV resulted in reduced viral replication (Figure 2).

The caprylic and capric acid combination (C8:C10) resulted in a 2.20 and 3.30 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) viral replication of Type II North American (P129) PRRSV at 200 and 300 µg/mL concentrations, respectively. Increasing concentrations of the caprylic and capric acid combination to 400 and 500 µg/mL resulted in 4.07 and 4.03 log reductions in Log_{10} $TCID_{50}$/mL viral replication, respectively ($P < 0.01$). The combination of caprylic and capric acid performed similar in Type I European (Lelystad) PRRSV resulting in a 1.90, 2.64, 4.14, and 4.10 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) viral replication at 200, 300, 400, and 500 µg/mL concentrations, respectively.

Reductions in viral replication, as determined by Log_{10} $TCID_{50}$/mL, were observed at 200 µg/mL for the combination of caprylic and lauric acid (C8:C12) with Type II (3.80 log reduction; $P < 0.01$) and Type I (3.44 log reduction; $P < 0.01$) PRRSV. Increasing the concentration of caprylic and lauric acid (C8:C12) to 300 µg/

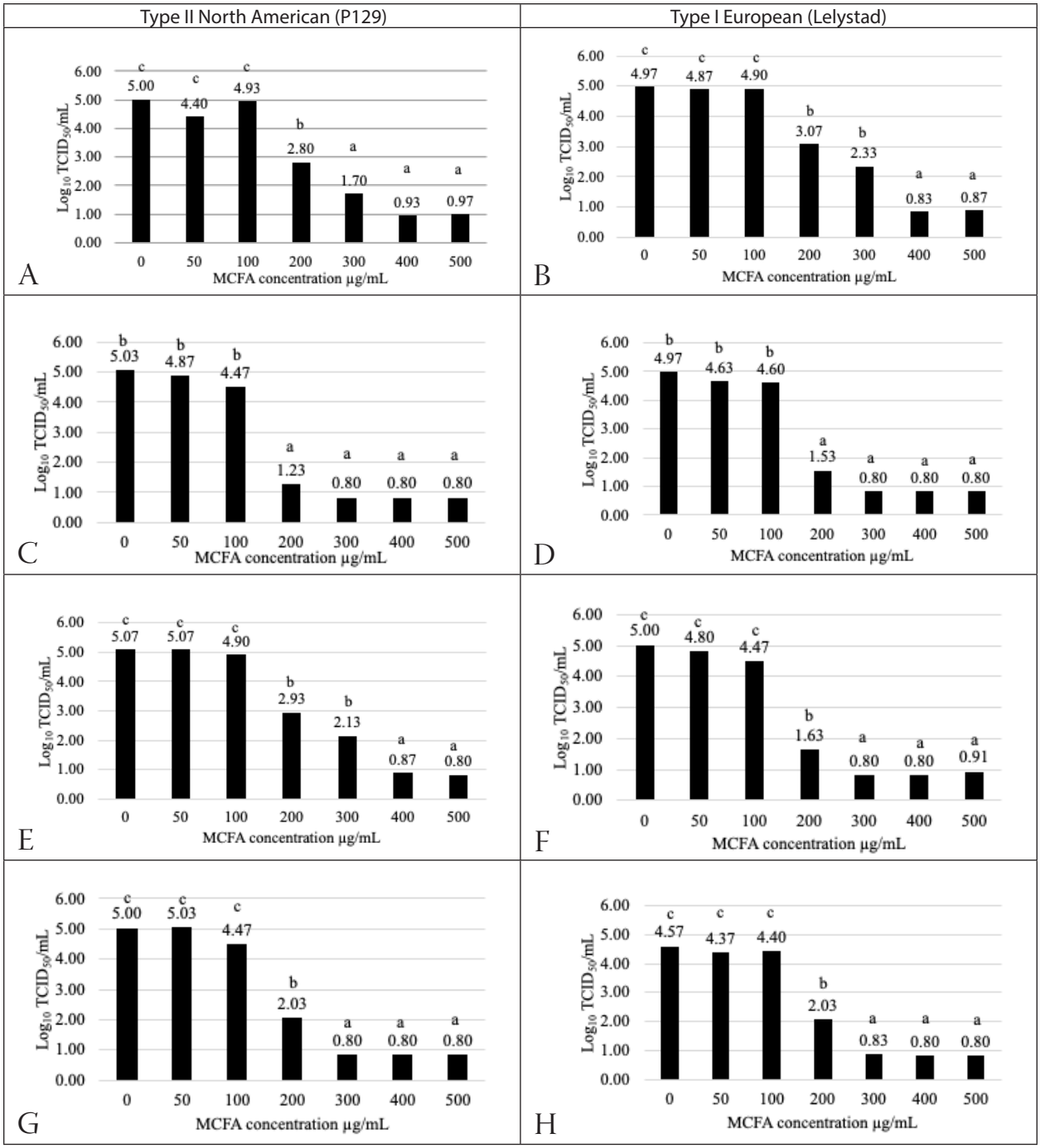

Figure 2. The effects of incubating MARC-145 cells with C8:C10 (panels A & B), C8:C12 (panels C & D), C8:10:C12 (panels E & F) or C8:C10:C12 (Panels G & H) prior to and after inoculation with Type II North American (P129) or Type I European (Lelystad) PRRSV on viral replication. [abc]Letters indicate differences ($P < 0.05$).

mL resulted in a 4.20 log reduction in Log_{10} $TCID_{50}$/mL viral replication with Type II ($P < 0.01$) and Type I ($P < 0.01$) PRRSV strains. Further increase in MCFA concentrations to 500 µg/mL did not further reduce viral replication.

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

The caprylic, capric, and lauric acid combination (C8:C10:C12) resulted in a 2.97 and 2.54 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) viral replication for Type

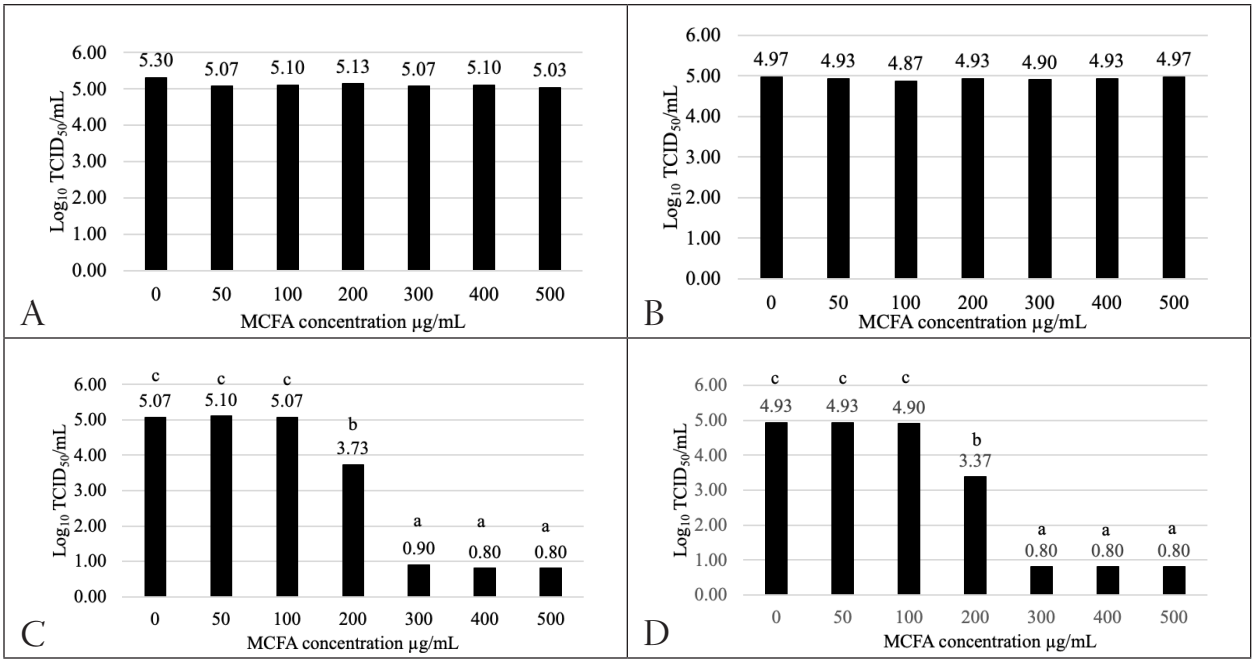

Figure 3. The effects of incubating Type II (Panel A) or Type I (Panel B) PRRSV with C8:C10:C12 prior to inoculation of MARC-145 cells resulted in no reduction ($P > 0.05$) in PRRSV replication as indicated by Log_{10} $TCID_{50}$/mL. Incubating MARC-145 with C8:C10:C12 prior to and after inoculation with Type II North American (P129) PRRSV resulted in 1.3 and 4.2 log reductions (Panel C: $P < 0.01$) in PRRSV replication at 200 and 300 µg/mL concentrations, respectively. The 400 and 500 µg/mL concentrations resulted in 4.3 log reduction ($P < 0.01$) in PRRSV replication as indicated by Log_{10} $TCID_{50}$/ml. MARC-145 cells incubated with C8:C10:C12 prior to and after inoculation with Type I European (Lelystad) PRRSV resulted in a 1.6 log reduction (Panel D: $P < 0.01$) in PRRSV replication at 200 µg/mL and a 4.0 log reduction ($P < 0.01$) in PRRSV replication at 300, 400 and 500 µg/ml as indicated by Log_{10} $TCID_{50}$/mL. ^{abc} Letters indicate differences ($P < 0.01$).

II North American (P129) and Type I European (Lelystad) PRRSV, respectively, when supplied at 200 µg/mL. Higher concentrations further reduced viral replication in both PRRSV strains. Viral replication incurred a 4.20 log reduction in Log_{10} $TCID_{50}$/mL ($P < 0.01$) at 300, 400, and 500 µg/mL for the Type II North American (P129) PRRSV. Viral replication of Type I European (Lelystad) PRRSV was reduced ($P < 0.01$) by a 3.74, 3.77, and 3.77 log reduction in Log_{10} $TCID_{50}$/mL at 300, 400, and 500 µg/mL respectively.

Mode of Action Evaluation

To determine if the effect of MCFA were mediated through MARC-145 cells, PRRSV or both, MARC-145 cells were seeded onto 96 well tissue culture plates and incubated at 37°C in a 5% CO_2 environment for 48 h. The MCFA combination of C8:C10:C12 was prepared and concentrations were made through serial dilutions in growth media to achieve 50, 100, 200, 300, 400, and 500 µg/mL concentrations. Viral dilutions were prepared via serial dilutions in growth media to achieve $1x10^{-1}$ to $1x10^{-8}$ viral infective particles/mL. Virus concentrations were then used to make a 50:50 mixture of virus and MCFA or virus and growth media to be used for inoculation. To test the effect of MCFA on MARC-145 cells, cells were exposed to MCFA concentrations, but virus was not. To test the effect of MCFA on PRRSV, virus was exposed to MCFA in a 50:50 mixture, but cells were not. All other procedures were as described above.

Results

Incubation of Type II North American (P129) or Type I European (Lelystad) PRRSV with MCFA (Figure 3) prior to inoculation did not affect viral replication ($P > 0.10$). However, incubation of MARC-145 cells with C8:C10:C12 prior to and after inoculation with Type II and Type I PRRSV did lead to a significant reduction in viral replication. Reductions in viral replication, as determined by Log_{10} $TCID_{50}$/mL, were observed at 200 µg/mL of C8:C10:C12 for Type II (1.34 log reduction; $P < 0.01$) and Type I (1.56 log reduction; $P < 0.01$) PRRSV. Higher concentrations (300-500 µg/mL) of C8:C10:12 led to a 4+ log reduction in Log_{10} $TCID_{50}$/mL replication for Type II and Type I PRRSV. These results indicate that the inhibition observed with MCFA is a specific effect on MARC-145 cells and not the PRRSV itself.

Conclusions

PRRSV infection results in increased inflammation and is immunosuppressive, therefore it has been hypothesized that providing MCFA through the diet during a PRRSV infection would help to support immune

function. MCFA are immunomodulatory and impact T cell signaling through GPR84 and monocyte differentiation through GPR40. MCFA C6, C8, C10, C12 have been reported to polarize naïve T cells to a Th17 and Th1 phenotypes and influence neutrophils through the activation of GPR84.

Results demonstrate that individual MCFAs of 8, 10 or 12 carbons or combinations of MCFAs were able to reduce viral replication in an in vitro PRRSV challenge model. In general, as MCFA chain length increased (from 8 to 12), the amount of MCFA needed to reduce viral replication decreased. The mode of action of MCFA have been reported as an effect on bacterial membranes or viral envelopes. However, the results of this experiment do not demonstrate a direct effect on the PRRSV. Future research is needed to investigate the mode of action through which MCFA reduce viral replication of PRRSV and results should be confirmed in a PRRSV challenge in pigs.

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