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An appraisal of the effect of nursing location on weaning weight of piglets and its dependence on the feeding of essential oils to sows

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An appraisal of the effect of nursing location on weaning weight of piglets and its dependence on the feeding of essential oils to sows

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THESIS
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A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

Shannon L. Dierking

Lexington, Kentucky

Director: Dr. Merlin Lindemann, Professor of Animal and Food Sciences

Lexington, Kentucky

2019

ABSTRACT OF THESIS

An appraisal of the effect of nursing location on weaning weight of piglets and its dependence on the feeding of essential oils to sows

The objective of these studies was to evaluate the effect of piglet nursing location on weaning weight, and its dependence on essential oil supplementation on sow and piglet performances.

Piglets that nursed anterior teat pairs had heavier weaning weights and higher gain for the lactation period. Additionally, piglet birthweight did not impact their overall teat selection and nursing location. These results provide some insight into the biological aspects of sow milk production, and implied that milk yield may vary between teat pairs along the udder line.

Supplementation of essential oils (EO) during late gestation and lactation had no effect on sow fecal dry matter (DM), immunoglobulin content of colostrum and milk, but it did increase the lactose content in milk from sows supplemented with EO, with an increase from 5.84% to 5. 97% (P = 0.04). There was an increase in sow weight loss during lactation (P = 0.002), and there was a significant effect on piglet birthweight, with sows supplemented with EO producing heavier piglets at birth, 1.56 kg in EO sows, compared to 1.49 kg in the control (CON) sows (P = 0.03).

Overall, piglet weaning weight is impacted by their selected nursing location along the udder line. Supplementation of EO may have limited effects on sow performance, such as fecal dry matter (DM) but may positively impact piglet birthweight. Furthermore, including EO into sow diets during late gestation and lactation can potentially impact the nutrient levels of sow milk.

KEYWORDS: Nursing piglet, Weaning weight, Essential oil, Sow, Milk.

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An appraisal of the effect of nursing location on weaning weight of piglets and its dependence on the feeding of essential oils to sows

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Dedicated to my father John. I wouldn't be on the path I am today without him involving me in all aspects of swine production from the time I was old enough to tag along to "help" with chores. For that, I will always be appreciative.

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Lastly, I would like to extend gratitude to my parents, John and Phyllis Dierking, because without Dierking Farms, and all the agricultural aspects they had me assist with, I would not be on the path I am today.

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Piglet weaning weight is an essential consideration within the swine industry. Typically, a heavier piglet at weaning is ideal, as that usually implies rapid growth after weaning and up to slaughter (Wolter and Ellis, 2001). Piglets that are heavier at weaning reach slaughter weight sooner than their lighter littermates (Mahan and Lepine, 1991). Typically, the first five pairs of teats (the anterior and middle pairs of teats along the sow udder line) have heavier wet and dry weights, as well as higher levels of protein and DNA (Kim et al., 2001). By gaining a clearer biological understanding of how, or if, milk production varies along the udder line can provide better insight into management practices that may assist piglets that are gaining slower than their littermates.

Essential oils (EO) are natural, bioactive compounds that derive from plants and have been known to have positive effects on an animal's health (Puvaća et al., 2013). Most essential oils are aromatic, volatile, and oily liquids, and are typically a mixture of various compounds (Zeng et al., 2015). Some have been shown to have antimicrobial, anti-inflammatory, antioxidative, and coccidiostatic properties, and may enhance digestibility in animals (Omonijo et al., 2018).

At or around farrowing, a common issue that sows face is constipation. This is due in part to the intestine becoming less active in preparation for the coming parturition (Le Cozler et al., 1999) and an increase in water absorption within the intestine in preparation for the beginning of milk production (Mroz et al., 1995).

Therefore, the objective of the current research was to evaluate the effect of piglet nursing location on body weight gain and subsequent weaning weight (Chapter 3) and

then measure the impact of essential oil supplementation on reproductive performance of lactating sows and their piglets (Chapter 4).

Chapter 2: Literature Review

2.1 Introduction

The projected world population is estimated to reach between 9-10 billion people by the year 2050, according to the report "World Population Prospects: The 2015 Revision" (Unies, 2015). With such a substantial increase in the world population, this prompts the looming question that the agriculture industry faces, "how will the agriculture sector feed the growing population in the future?" With regards to increasing pork supply, increasing the number of pigs produced per sow each year is a logical part of the answer. Granted, with the fixed land space available for agriculture production, and the dilution effect of fixed production costs, other aspects of pork production must also be improved, such as growth rate and efficiency.

2.2 Changes in Industry Numbers

Increasing litter size has been an on-going goal within the industry. Early improvements in litter size were achieved with better management and nutrition. Recently, effective implementation of genetic selection for litter size from the maternal line (Rutherford et al., 2013) has increased overall numbers in litter size for total born alive from an average of 10.34 in 2004 to 12.96 in 2018, resulting in an increase in total weaned from 9.10 to 11.34 (PigCHAMP, 2018). There are several measures of litter size to also take into consideration: total born, stillborn, mummies, liveborn, and liveborn/sow/year (PigCHAMP, 2015). While there are positive benefits that can result from an increase in litter size, as litter size increases there is a strong probability that pre-weaning mortality will also rise. From 2004 to 2013, pre-weaning mortality increased by 0.91%, from 12.47 to 13.72% (PigCHAMP, 2015). This increase in pre-weaning mortality is impacted by the

number of pigs with birth weights under 1 kg. Piglets weighing less than 1 kg may struggle to thrive during lactation and face a higher risk of pre-weaning mortality.

Lactation presents unique challenges for the sow; following the birth of the litter the sow must provide nutrient-rich colostrum and then a large quantity milk for each of her piglets to facilitate growth for the remainder of the lactation period. Maintaining a high level of output can take its toll on the sow's nutrient stores within her body. Nutrition and litter size all impact a sow's milk production and her point of peak lactation milk production, which will impact subsequent litter gain; it is essential to provide the sow with diets that have an overall positive effect on the nutrient composition.

While the composition of sow colostrum and milk have been studied and documented, the effects of piglet nursing location on individual piglet gain and subsequent weaning weight have not been evaluated in swine. Consequently, a review of the current literature with regards to milk production and composition in sows and piglet nursing behavior is a logical starting point to address these questions.

2.3 Mammary Gland Development

Sow mammary glands are in two parallel rows that sit along the ventral body wall, from the thoracic region to the inguinal area, and is attached by adipose and connective tissue. Each gland is separate and distinct from adjoining glands and has one teat with two separate teat canals (Turner, 1952). Each canal contains a self-contained duct and glandular system (Hughes and Varley, 1980). In utero, mammary tissue is derived from the ectoderm in the embryo, and differentiation of the udder becomes apparent in the very early embryonic stage, in which two parallel lines of ridges form, which are known as "milk lines." These nodules form into mammary buds, which serve as the progenitor of a teat (Farmer, 2015).

Within the teats, the accumulation of mammary tissue and DNA is indicative of cell growth. The accumulation of tissue is relatively slow until approximately 90 days of age in the gilt. The mammary glands undergo three stages of cyclical changes during each gestation/lactation cycle. These stages are mammogenesis, lactogenesis, and involution. Mammogenesis is the process of mammary tissue growth and is thought to begin at the onset of puberty or estrous cycles in gilts. Parenchymal growth within the mammary gland is stimulated by an increase in estrogen production (Farmer, 2015). Following an estrous cycle, development and ovulation of the follicles stimulate the formation of corpora lutea which regress after 12 days. Corpora lutea contains relaxin, which is released into general circulation when they regress (Farmer, 2015). Relaxin stimulates parenchymal growth and may have a direct impact on the milk production potential for each mammary gland, due to its stimulation of parenchymal cells.

Until late gestation, mammogenesis occurs slowly. Before the final stages of mammogenesis, there are significant increases in estrogen, relaxin, and prolactin. These hormone increases occur at a high rate during the last 30 days of pregnancy (Farmer, 2015). Prolactin is considered the essential hormone for the final stages of mammary gland development, as it stimulates both mammogenesis and lactogenesis. This stimulates gland development and production of colostrum and milk. Without the release of prolactin, the sow would struggle to feed the piglets due to low milk production.

2.4 Production of Colostrum and Milk

Colostrum yield is highly variable between individual sows, even within the same breed of sows and raised in similar conditions of housing and feeding (Quesnel, 2015). Lactogenesis is defined as occurring when the rise of lactose in the mammary glands, which

also correlates to an increase in the lactose concentration in plasma (r = 0.88, P < 0.01) (Hartmann et al., 1984). Before this analysis, lactose was only measured in mammary secretions in rats, rabbits, sheep, and women. This rise in lactose can occur anywhere between 2 and 7 days before parturition. The mammary gland is the only organ that undergoes most of its development after parturition, due to the resulting increase in cell numbers from piglet suckling (Panzardi et al., 2013). Colostrum is secreted in small amounts during the initial period of parturition and then increases during the first 24 hours after parturition. Transient milk occurs after colostrum until approximately day 4 of lactation, and mature milk is defined as the secretions that occur after day 10 (Csapo et al., 1996; Klobasa et al., 1987). There are several components that make up the majority of milk and colostrum composition.

2.5 Composition of Colostrum and Mature Milk

Colostrum is defined as the first secretion of milk from the mammary glands within the first 24h of life (Farmer, 2015). It is essential for the piglet's early survival, as it provides the energy needed for thermoregulation in a cold environment. Colostrum contains high levels of nutrients, and also gives the piglet immunoglobulins. Due to the epitheliochorial nature of the placenta, the piglet is unable to receive passive immunity transfer from the sow. At birth, the piglet must absorb immunoglobulin macromolecules in colostrum prior to gut closure (Sjaastad et al., 2012). Colostrum contains three primary immunoglobulins (IgG, IgA, and IgM), which provide the piglet with immunity (Farmer, 2015). The milk produced during the later stages of lactation has significantly lower levels of immunoglobulins. The components of colostrum and milk, which are fat, protein, and lactose, as well as the production of each, are discussed in the subsequent sections.

2.5.1 Fat

Porcine milk is typically higher in fat content than that of most other mammals (Table 1.1). It has a higher overall milk concentration of the fat component of 8.2% as compared to cattle, horses, sheep, and humans. The fat levels in it account for 40-60% of the total energy in colostrum provided to newborn piglets during the first day of life (Alexopoulos et al., 2018). The fat content within colostrum and milk are crucial for piglets, as it is the primary energy source for thermoregulation (Hurley, 2015). During mid- and late-lactation, the piglet growth rate increases significantly. The sow accounts for this increased need in energy for the piglets, and milk produced after the first seven days of lactation has the highest fat content at 9.8% (Theil et al., 2014). Specifically, the fat content of milk typically reaches a plateau after day 7 of lactation and remains constant until weaning, from 5.1% during early parturition, to a constant level around 8.2% on D 17 of lactation (Table 1.1) (Theil et al., 2014).

Table 1.1 Milk composition of different species[1]

Species	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Total Solids (%)
Camel	4.9	3.7	5.10	0.7	14.4
Cattle					
Holstein	3.5	3.1	4.9	0.7	12.2
Jersey	5.5	3.9	4.9	0.7	15
Deer	19.7	10.4	2.6	1.4	34.1
Elephant	15.1	4.9	3.4	0.76	26.9
Horse	1.6	2.7	6.1	0.51	11
Human	4.5	1.1	6.8	0.2	12.6
Pig	8.2	5.8	4.8	0.63	19.9
Rabbit	12.2	10.4	1.8	2.00	26.4
Sheep	5.3	5.5	4.6	0.9	16.3

[1]Adapted from Zhang et al. (2018).

2.5.2   Protein

In colostrum, the protein content is highest at parturition and reduces considerably during the first 24 hours of lactation, by as much as 50%. This decrease is consistent with immunoglobulin secretion, which is highest during parturition but falls over the next 24 hours. However, other proteins that are used for nutritional purposes, such as casein and alpha-lactalbumin are low in colostrum initially and then increase during the first week of lactation (Quesnel et al., 2015). The casein fraction is important to the piglet due to its amino acid content to help meet the nutritional requirements of the nursing piglet (Aumaitre et al. 1978). Casein is also essential in stomach clotting, which governs the emptying of the stomach of the colostrum and subsequent milk protein (White et al. 1969).

The primary protein components in colostrum are the immunoglobulins. The immunoglobulin concentration within colostrum is vital for piglet survival. Piglets are born immunologically naïve, as the sow is unable to transfer antibodies in utero to the piglets via the placenta (Alexopoulos et al., 2018). Immunoglobulin transfer occurs in two primary ways, either through serum transfer or via de novo synthesis by mammary tissue. Immunoglobulin production and transfer differ in mature milk, as it is thought that production occurs by the mammary glands themselves (Curtis, 1973). The three most common immunoglobulin isotypes are IgG, IgA, and IgM. Concentrations of immunoglobulins are highest in colostrum during the first several hours postpartum. IgG is the principal constituent of colostrum but decreases rapidly during the first 24 hours. By hour 12 postpartum, IgG concentrations can decline by up to 50% and continues to reduce through 48 hours postpartum. In contrast to colostrum, IgA becomes the principal immunological constituent in mature milk. This shift in immunoglobulin level reflects the

changing need of the piglets, as total protein absorption gives way to localized immune protection within the gut (Darragh and Moughan, 1998). Passive immunity is only able to occur over a short window of time. A piglet's gastrointestinal tract (GIT) will undergo "gut closure" in which antibodies are no longer able to pass between the intestinal cells and enter the vascular or lymph systems, typically around 24 hours postpartum (McKay and Rahnfeld, 1990).

As previously mentioned, IgG is the principal component of colostrum. It is reported by some to be the most critical globulin during the first few weeks of life to help sustain both immunity and growth (Kielland et al., 2015; Markowska-Daniel and Pomorska-Mol, 2010). However, this contrasts with work published by Gaskins and Kelley (1995) which stated that IgG antibodies typically have limited effectiveness against pathogens the piglet encounters during the nursing phase. IgG can be produced by mammary tissue, but the majority of IgG is transferred from the serum.

IgA is the most prominent immunoglobulin in both transient and mature milk produced by the sow. IgA acts as a barrier at the mucosal level (Markowska-Daniel and Pomorska-Mol, 2010) and provides short term protection against bacterial infections (Gaskins and Kelley, 1995). The IgA immunoglobulins can act in this way as they are only partially degraded within the intestinal tract.

IgM is found in the smallest concentration in both colostrum and milk (Farmer, 2015). IgM is typically found in the blood and immature B-cells. Once the B-cells mature, they begin to produce other immunoglobulin isotypes (ex: IgG, IgA). IgM appears first when the body is exposed to an antigen (Farmer, 2015).

2.5.3   Lactose

The most prevalent sugar in colostrum and milk is lactose. Compared to other components found in colostrum and milk, it has the smallest variation. On average, lactose content in both colostrum and mature milk is between 3-4% (Atwood and Hartmann, 2009). Glucose levels in blood influence the levels of lactose in milk. Approximately 59% of plasma glucose transported into the mammary gland is used to develop lactose levels found in milk (Zhang et al., 2018).

2.6 Lactation Milk Yield

Between 1935 and 2010, milk yield has increased from approximately 4 to 11.50 kg/d (Kim et al., 2013). Helping the lactating sow to reach her peak genetic milk production potential to provide the most milk to her piglets is crucial in preventing pre-weaning mortality and assisting piglets to achieve a heavier weaning weight. Piglets weaned before 21 d of age typically only consume milk, so milk yield is a critical limiting factor for their growth rate. Besides nutrition of the sow, the age of the sow also has an impact on the average milk yield. A first-parity gilt will typically produce less milk throughout lactation than a parity two sow and beyond. This is due in part to the development of the mammary glands along the udder line. The number of cells present in the mammary gland influence the milk yield from that teat. Mammary gland size is directly correlated to its potential milk yield (Nielsen et al., 2001). Teats that have been nursed previously will contain a heavier wet weight, as well as more DNA and RNA per teat (Farmer et al., 2010). On average, a first-parity gilt will produce around an average of 8 kg/d milk yield, with an increase to sixth-parity sows at 12 kg/d (Whittemore, 1990).

A variety of different factors can impact milk production during lactation. Sow breed can affect milk yield. Chinese-derived sow breeds produce more milk than sows from common European descent (i.e., Landrace, Large White), but they both provide more than meat-type breeds such as Duroc or Pietrain (Farmer, 2015). Litter size and suckling intensity are a major determinant of sow milk yield, as the number of suckled mammary glands is proportional to milk production (Auldist et al., 1998). Within the modern production system, continuous loud noise has resulted in less teat stimulation, which results in a decrease in milk output (Algers and Jensen, 1991). This may be due in part to the understanding that sows within a farrowing room will synchronize their nursing with other litters. They hear auditory stimulus from the other animals around them, and thus, nursing throughout the room occurs (Rzezniczek et al., 2015). Therefore, a continuous loud noise may inhibit the synchrony of nursing within a farrowing room.

The mammary epithelium impacts milk yield, particularly the number of mammary alveolar cells present within a gland. The growth of a gland is affected by the anatomical location on a sow. Glands that are located in the middle part of the udder (typically known as the 4th and 5th pair) grow faster during gestation and generally are larger than those in both the anterior (1st, 2nd, and 3rd pairs) and posterior (6th, 7th, and 8th) locations at farrowing (Ji et al., 2006). However, during lactation, teats that are more anterior grow faster than the rest (Kim et al., 2001). This could be due to piglet choice, as piglets typically imprint on the more anterior teats at the beginning of lactation, before nursing teats located posteriorly. This could be a result of the initial selection process following parturition. Piglets begin to establish dominance by sampling multiple teats. Heavier piglets can defend their teat from

their smaller counterparts, which may explain why smaller piglets typically end up nursing posterior teats that are small and not as productive (Klobasa et al., 1987).

2.6.1   Individual Teat Variation

A sow's udder line can have anywhere from 12-16 teats, depending on spacing. As mentioned previously, the number of alveolar cells present within a mammary gland plays a crucial role in milk yield potential. The first five pairs of teats (the anterior and middle pairs of teats along the sow udder line) have heavier wet and dry weights, as well as higher levels of protein and DNA (Kim et al., 2001). Blood flow to the teat pairs also has an impact on milk production. The arterial, venous, and lymphatic circulation of the sow mammary glands are provided on each side of the ventral midline by a network that extends longitudinally from the axillary to the inguinal regions (Schummer et al., 1981). Unlike ruminants, the mammary glands of sows receive blood from each side of the udder through several arteries (Busk et al., 1999). There is an external pudic artery that runs downward and descends through the inguinal canal where it divides into branches. The arteria epigastrica cranialis supplies the anterior mammary glands for pairs 1 to 5, and then branches of the arteria pudenda externa, arteria epigastrica, caudalis, and arteria epigastrica superficialis supply the posterior pairs of glands (Trottier et al., 1995a). Mammary blood flow can be affected by postural changes, milk demand, day in lactation, and environmental temperature (Farmer, 2015). The differences in blood flow to different teats may impact nutrients to the piglet. This would provide some explanation to recent work published by Lannom et al. (2018), who found that the first two pairs of teats produced higher quality colostrum and more mature milk than the last two pairs of teats.

2.7 Changes in Litter Size

As previously stated, to increase production output, producers have begun taking measures to increase litter size. Sows are now producing larger litter numbers than those 20 years ago (MLC, 1979, 1999). In the U.S., from 2004 to 2018, the average number of piglets born alive per litter has increased from 10.34 to 12.96 (PigCHAMP, 2018). Litter size is one of the significant factors that influence milk production during lactation (Whittemore, 1993), as well as litter weight gain (Kim et al., 2000). As litter size increases, sow milk yield increases linearly. However, milk intake per piglet decreases as increased competition decreases availability for the individual piglet (Whittemore, 1993). The increased nutritional demands that come with nursing a larger litter results in increased removal of nutrients from body tissues (Jones and Stahly, 1999). As litter size increased, protein mobilization from the sow's carcass, gastrointestinal tract (GIT), and reproductive tract increased linearly (Kim and Easter, 2001). Maintaining nutrient availability for the sow is essential in managing her body condition score (BCS), which is an assessment of the amount of fat and muscle that cover the bones of an animal, regardless of body size. It is important the sow maintains a healthy BCS during lactation.

2.8 Birthweight Variation

In recent years, the selection for improved prolificacy has indeed resulted in the previously described increase in litter size. However, an increase in litter size causes a detrimental decrease in birth weight (BW) within a litter (Roehe, 1999). Additionally, larger litter size can result in greater variation of piglet birth weights, which often results in higher piglet mortality (Quiniou et al., 2002). A piglet with a low birthweight can struggle throughout the rest of the lactation period. Lighter BW piglets possess less body

energy stores, which could make them more susceptible to temperature variation, and reduce their ability to thermoregulate their body temperature (Le Dividich, 1999). Smaller piglets may also be pushed down farther along the sow's udder line, which could result in a decreased intake in colostrum. This could result in a poor acquisition of passive immunity and an overall reduced nutritional status for the piglet. Lighter BW piglets typically have an overall lower performance in lactation than their heavier counterparts (Quiniou et al., 2002). Heavier pigs win more teat disputes (Scheel et al., 1977), gain more weight (Milligan et al., 2001), and experience lower mortality rates (Tuchscherer et al., 2000). Lighter BW piglets will struggle in the subsequent grow-finish period, which results in a greater length of time to reach market weight (Mahan, 1993). Finding the ideal birthweight that helps maximize litter size while giving the neonatal piglets the best opportunity at pre-weaning survival is crucial to the industry. Smith et al. (2007), measured the effect of piglet survivability to 42 days post-weaning based off nine birthweight categories. Each category incrementally increased (four categories) or decreased (five categories) by 0.5 standard deviations (SD) from the birthweight means, from 0.77 to 2.24 kg. Table 1.2 is an adaptation of their results, which shows that maximum piglet survival (93.8% to 97.1%) from birth to weaning has been reported to occur for piglets with a mean birthweight of 0.98 to 1.30 kg ± 0.50 kg SD with the poorest survivability for piglets with a mean birthweight of 0.77 ± 0.50 kg SD (71.2% survivability) (Table 1.2). This disagrees with work by Gardner et al. (1989) which separated piglet birthweight into 9 categories and concluded that increases in birthweight were associated with increased odds of survival to weaning at 21 days, with maximum survival in the heaviest birthweight category.

Table 1.2. Effect of mean piglet birthweight on survivability to 42 days post weaning adapted from Smith et al. (2007).

Birth-weight category*	No. of piglets	Birth weight (kg)				Survival (%)
		Minimum	Maximum	Mean	SD	
1	59	0.57	0.87	0.77	0.08	71.2
2	139	0.88	1.04	0.98	0.05	97.1
3	259	1.05	1.21	1.14	0.05	93.8
4	405	1.22	1.38	1.30	0.05	95.6
5	617	1.39	1.55	1.47	0.05	79.6
6	566	1.56	1.72	1.64	0.05	82.5
7	407	1.73	1.89	1.80	0.05	78.4
8	273	1.90	2.06	1.96	0.05	87.2
9	168	2.07	2.85	2.24	0.16	86.3

*Each piglet was individually identified and weighed within 24 hours of birth. Birth-weight categories incrementally increased or decreased by 0.5 SD (0.16 kg) from the birth weight mean (1.57 kg). Pigs were weighed at an average of 15 days of age (weighed at 14, 15, or 16 days) or an average of 20 days (19, 20, or 21) days.

2.9 Pre-weaning Growth Rate

As previously mentioned, piglet birthweight is influential in overall piglet weaning weight. However, milk consumption during lactation is essential for piglet growth and development. Thus far, much of the literature review has discussed causes that affect the sow and the subsequent nutritional make-up of the colostrum and milk, but there are behavioral aspects of the piglets that also play a role in their ability to grow throughout the lactation period.

2.9.1   Early Lactation Nursing

Once a piglet is born, it begins to look for a teat (Fraser et al., 1995). Typically, the first piglet has the hardest time finding the sow's udder, with subsequent piglets locating

them at a faster rate. The first piglet moves along the udder line by maintaining contact with the sow. The sow may also communicate with the piglet, which can have a positive influence in attracting pigs (Skok et al., 2007). During the first 8 hours following parturition, piglets will suckle multiple teats along the udder. Piglets may fight or push littermates out of the way to obtain other teats (Farmer et al., 2015). Within this period, piglets may suckle up to 7 different teats without establishing a preference for a specific one. The establishment of teat dominance is thought to take between 3-7 days, during which time the piglet shows a progressive tendency to confine themselves to one area of the udder, slowly narrowing their preferred area to the final, definitive teat (Rossillon-Warnier and Paquay, 1984). This contrasts work by other authors, who state that between 5% and 50% of piglets have established ownership of one specific teat by the end of day 1 of life (de Passile et al., 1988; Puppe and Tuchscherer, 1999).

Piglet competition for teats occurs in every litter. Piglets will use their size, as well as their sharp canine and incisor teeth for biting the competition when trying to determine teat order (Farmer et al., 2015). There is a correlation between birth order and success in winning teat disputes. Piglets that are born earlier will sample more teats and tend to win more teat disputes than their later-born littermates (de Passille and Rushe, 1988). Piglets that are unable to acquire a teat early in lactation, which may occur for lighter-birthweight piglets, will end up expending more energy trying to displace littermates from their teat (Farmer et al., 2015). Due to this, litter size has a direct impact on a piglet's ability to select a teat and thrive. Heavier piglets will typically nurse the more anterior teats, with lighter piglets ending up on the posterior end of the udder. Rear teats produce less milk overall than anterior or mid-section teats (Skok et al., 2007; Pluske and Williams, 1996).

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2.9.2   Methods to Estimate Milk Intake

The ability to calculate sow milk yield or piglet milk intake has been studied extensively over the past years. Being able to understand these components are important aspects of animal husbandry. During lactation, energy, and amino acid intake of the sow partitions within her body to milk constituent synthesis (Noblet et al., 1989), as well as her tissue deposition that maintain a healthy BCS. There are several methods used to calculate milk yield, but all have their limitations. The weigh-suckle-weigh (WSW) method has several different methodologies used (Salmon-Legagneur, 1956; Speer and Cox, 1984). The WSW method is based on weights of piglets immediately before and after nursing of the sow. Speer and Cox (1984) observed hourly nursings for 9 consecutive hours at D 14 of lactation, and the sum of the piglet weight gains is recorded as the amount of milk consumed (Pettigrew et al., 1985). Perhaps the method most commonly used is one introduced by Noblet and Etienne (1986). Piglets are removed from the sow on D 1, 5, 9, 13, 17 and 21 of lactation. Ten suckling sessions are measured with 72-minute intervals between each nursing session. Piglets were encouraged to urinate and defecate before each session and litter weight gain was corrected for weight losses as a result of water evaporation between weighings (Noblet and Etienne, 1986). The first two nursing sessions consisted of an adaptation period, as these values were consistently lower than the others. The other eight sessions were used to calculate daily milk production. Additionally, heat production of the piglets was measured on the same day in a confinement chamber. This value was subtracted from ME intake as milk to estimate litter energy retention. Composition of the sow milk was determined on each day following milk production measurements. An aspect that could influence this method is that stress could play a role

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in output and subsequent nutrient composition because the piglets are kept isolated from the sow between nursings, it may result in an artificial suckling frequency and subsequently cause a reduction in overall milk production values.

To offset the stress effects that could occur from the sow's isolation from her litter, isotope dilution techniques were introduced, using either tritiated water or deuterium oxide (D_2O) (Pettigrew et al., 1985, 1987; King et al., 1993; Toner et al., 1996). In this method, piglets are injected with an isotope of water, D_2O, and then the degree to which total body water is diluted by milk consumption is measured by CO_2 output (referred to as the breath test) (Theil and Kristensen et al., 2007). This assumes that milk or colostrum is the piglet's only source of water, and from that, one can calculate the amount of milk consumed if the composition of milk is known. From the chemical composition, potential metabolic water stored can be determined, which is based on the assumption that retention of DM in piglets is equal for deposition of both fat and protein (Theil and Kristensen et al., 2007). An advantage of this method is that it does not disrupt the normal maternal-offspring relationship (Pettigrew et al., 1985).

From techniques mentioned previously, several researchers have introduced different mathematical models to estimate milk yield. Noblet and Etienne (1989) developed a model that predicts average milk yield from litter gain. However, this model only provides an average milk yield, which is inconsistent with research that shows that milk yield changes throughout lactation. Other authors have developed models to describe the lactation curve (Whittemore and Morgan, 1990; Walker and Young, 1992). Another method used in today's research applications is the milk production curve introduced by Hansen et al. (2012). The average milk yield of a sow is impacted by several influences

such as parity, litter size, and litter gain. A database was created that contains data on litter size, litter gain, dietary protein and fat content, milk yield, and composition measured beyond d 1 of lactation, building off different methods of milk yield determination in peer-reviewed publications. It built off the Wood curve used to determine a lactation curve in cattle (Hansen et al. 2012). The equation for the lactation curve is below (adapted from Hansen et al. 2012). Where $y(t)$= milk yield (kg*d) at the time (t) after parturition (d).

$y(t) = a * t^b + \exp(-c * t)$ where:
$a = \exp(1/3*(-ly_{20} * \log(128/27) - 3*\log(20)*ly_{30} + 5*\log(20)*ly_{20} - 2*\log(20)*ly_{5} +$
$4*ly_{5}*\log(128/27) + 12*ly_{30} - \log(5) - 20 - \log(5)*ly_{20} + 8*\log(5)*ly_{5})/\log(128/27))$
$b = -(3*ly_{30} - 5*ly_{20} + 2*ly_{5})/\log(128/27)$
$c = 1/15*(ly_{5}*\log(128/27) - ly_{20}*\log(128/27) -$
$3*\log(20)*ly_{30}$
$+ 5*\log(20)*ly_{20} 2*\log(20)*ly_{5} + 3*ly_{30}*$
$\log(5) - 5*\log(5)*ly_{20} + 2*\log(5)*ly_{5})/$
$\log(128/27)$

In this equation, $y(t)$ = milk yield (kg*d) at the time (t) after parturition (d). The parameters are ly_{5}, ly_{20} and ly_{30}, which represent the natural logarithm of the milk yield at d 5, 20, and 30 in lactation.

Creating a framework using a database with a wide variety of information can allow a user to ascertain a value for any time point during lactation.

2.10   Weaning Weight

In a natural environment (i.e., in the wild), piglets are weaned from their mothers between 16 and 18 weeks of age (Jensen and Recen, 1989). In the current practical farm system, piglets are weaned between 2 to 6 weeks of age before their digestive systems are fully developed (Bailey et al., 2005). The piglet must adjust to the abrupt interruption of its primary source of nutrients (sow milk), and adapt to less digestible, plant-based dry

diets that contain complex protein and carbohydrate sources (Cranwell, 1995; Lalles and Awati, 2007). When piglets no longer have access to sow milk, they lose the availability of maternal IgA, which is used to control pathogens that are colonized in the gut bacteria (Kelly and King, 2001). After weaning, the pathogens can utilize the chyme within the stomach to colonize and proliferate (Pluske et al., 1997). The stress associated with weaning, which includes separation from the sow, the movement to a new environment, and introduction to a new diet can result in nutritional stress and an overall reduction in piglet growth in the initial days following weaning (Blecha and Kelley, 1981). Typically, a heavier weaning weight implies rapid piglet growth after weaning and up to slaughter (Wolter and Ellis, 2001; Smith et al., 2007). There is a linear relationship between the weaning weight of the piglet and average daily gain in the post-nursery period (Cabrera et al., 2010). Piglets that are heavier at weaning reach slaughter weight sooner than their lighter counterparts (Mahan and Lepine, 1991). The potential decrease in growth that piglets experience as a result of lighter weaning weight and associated post-weaning stress and weight loss, can result in increased cost to the industry, which includes a more extended feeding period before reaching market weight, slower turnover of the facility, and possible requirements for specific nutrient supplementation for weaker pigs.

Thus far, the focus has been on the biological aspects of milk production within the sow and its subsequent effect on her piglets. Beyond that, there are also nutritional components within the diet that have an impact on overall milk yield and composition.

2.11 Sow Feed Intake

Proper nutrition for the sow during pregnancy is important to the overall health of both the sow and her piglets. The first month of gestation establishes a successful

pregnancy and allows the litter size to be determined based off of the number of viable embryos (Farmer, 2015). During pregnancy, maintenance of the sow and the growth of the embryos are considered to receive priority for the nutrients. Once those needs are satisfied, the extra nutrients are deposited in maternal tissues (Farmer, 2015). Understanding individual sow feed requirements is an important aspect of management. Increased sow feed intake during gestation would allow for growth of the fetus, as well as deposition of body fat and protein, but could result in increased weight loss during lactation (Cox and Cooper, 2001). Weldon et al. (1994) reported that sows that were fed ad libitum access during the final 40 d of gestation had an overall reduction in voluntary feed intake during lactation. While providing ad libitum access throughout gestation, may have negative implications on feed intake during the lactation period, an increase in feed intake, specifically in late gestation may have a beneficial effect. Mahan (1998) reported that when gestation feed intake increased by 0.13 kg (or 450 kcal ME) larger litter size resulted ($P <$ 0.01) with no effect ($P > 0.15$) on lactation feed intake.

Once the sow reaches the lactation period, maximizing voluntary feed intake is essential. A decrease in feed intake may be a significant contributor to a greater reduction in BW and greater back fat loss (Koketsu et al., 1996; Eissen et al., 2003; Anil et al., 2006). A sow's feed intake during the lactation period may also impact her future reproductive performance. Koketsu et al. (1996) analyzed farm records for 20,296 lactating sows across 30 commercial farms in the US. He demonstrated that sows that experienced a rapid increase of feed intake during lactation had significantly shorter ($P < 0.01$) weaning-to-first service interval and weaning-to-conception interval than sows with lower feed intake.

2.12   Dietary Additions

As discussed above, a reduction in feed intake during the lactation cycle can have a negative effect on the sow; additionally, another component that must be brought into consideration is individual dietary additions or alterations that may influence the performance of both the sow and her litter. Supplementation of fat in the diet may increase the output of fat and energy in milk, which may influence progeny performance (Lauridsen and Danielsen, 2004). In a trial that fed 175 sows a diet of either control (CON): 0% added dietary fat, or one of 5 treatment diets containing 8% dietary fat of either animal fat, rapeseed oil, fish oil, coconut oil, palm oil, or sunflower oil, had a positive impact on the daily output of fat in milk compared to the CON group with the inclusion of fat in the diet, as well as the different dietary sources ($P < 0.01$), with the CON group containing 6.5% fat, compared to levels of 7.1% (animal fat), 6.7% (rapeseed oil), 6.5% (fish oil), 7.5% (coconut oil), 7.1% (palm oil) and 6.9% (sunflower oil) . Pettigrew et al. (1981), found that there was an increase in colostral and milk fat concentration as a result of fat supplementation of the sow's diet. In this instance, animal fat or corn oil were added to the diets at a rate of 6%, with control sows producing milk with 6.50 % fat, compared to the animal fat and corn oil, which produced levels of 6.78% and 7.88% fat.

Feed additives, such as antibiotics, fed at a sub-therapeutic level have been utilized within the swine and poultry industry in past decades. They provided an improved growth rate and feed efficiency, and helped decrease overall morbidity (Zeng et al., 2015; Cromwell, 2002). In recent years, following the ban of antibiotic growth promoters (AGP), research has shifted to studying essential oil supplementations as an alternative to antibiotics in swine and poultry production. Essential oils are natural bioactive compounds that are derived from plants and have been known to have positive effects on an animal's

health (Puvaća et al., 2013). Most essential oils are an aromatic, volatile, and oily liquid, and are typically a mixture of various compounds (Zeng et al., 2015). They have been shown to have antimicrobial, anti-inflammatory, antioxidative, and coccidiostatic properties, as well as enhance digestibility (Omonijo et al., 2018). Essential oils consist of two major types of compounds, terpenes, and phenlypropenes. Terpenes are divided into subcategories based on the number of 5-carbon building blocks they possess (Omonijo et al., 2018). One type of essential oil is mastic gum, derived from the Chios Mastiha tree, grown along the Mediterranean coast (Association, 2014). Kroismayr et al. (2006) reported that essential oil supplementation of oregano, thymol, or carvacrol may increase the overall feed palatability and intake with the enhanced flavor and odor. However, this has not been a consistent observation when essential oils have been added to weaned pig diets: in contrast, supplementation of nursery pig diets with supplementation of oregano oil at 25, 50, and 100 g per metric ton, had no impact on ADFI (Neill et al., 2006) neither did oregano supplementation at 2, 4, and 8 g/kg feed (Stelter et al., 2013). One common essential oil as an addition to sow diets is oregano essential oils (OEO). It is extracted from plants by steam distillation. Supplementation of this essential oil may have a positive impact on sow feed intake. In a trial which supplemented sows 15 mg/kg of oregano (OEO) during gestation and lactation, ADFI was not different ($P > 0.10$) except during week 3, when sows fed the oregano diet had an ADFI of 6.46 kg compared to 6.03 kg of sows in the control diet group ($P = 0.007$) (Tan et al., 2015). In a study involving 2,100 sows, Allan and Bilkei (2005), found that supplementation of sow diets with 1 g/kg blend of OEO, had higher voluntary feed intake. However, this is not consistent across all trials, as Ariza-Nieto (2011), reported that sows fed a diet of 250 mg/kg of OEO did not impact ADFI in either

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gestation or lactation (P > 0.50). This agrees with a report by Mellencamp et al. (2009), which found that oregano diet supplementation did not increase sow feed intake. The different results from the described trials may be impacted by the supplementation level of the essential oil.

Essential oil supplementation may also impact the fat content of milk during lactation. Work by Ariza-Nieto et al. (2011), fed sows a diet containing 250 mg/kg of oregano essential oils. While the supplementation had no effect on gross energy (GE), crude protein (CP), GE:CP, GE:fat concentration in sow milk (P > 0.05), there were reductions in fat percentage of milk on d 7 (P < 0.05) and d 14 (P = 0.07) in the oregano supplementation group, and there was a trend (P = 0.10) for greater milk intake of the piglets in the supplemented groups. While there may be a decrease in nutrient levels, there may be a positive impact on milk yield. Work by J. Khajarern and S. Khajarern (2002), fed lactating sows OEO at a rate of 0.025% in the sow feeds and impacted overall daily milk yield of the sow (9.53 kg/d CON vs. 10.44 kg/d OEO). It's important to understand that dietary additions to sow diets may have an impact on the nutrient composition of her milk during lactation and must be taken into consideration.

Another common feed addition in sow diets is fiber. Dietary fiber is defined as the indigestible portion of a feedstuff that is derived from plants (Jarrett et al., 2018). It plays a key role in swine diets for its impact on physiological processes, such as gut fill and gas production following fermentation in the colon. There are many different types of fiber products available that are used in a variety of livestock diets, which include distillers dried grains, soybean hulls, wheat bran, sunflower meal, and beet pulp. These fiber sources include both non-starch polysaccharides, including pectins and cellulose, as well as

oligosaccharides and starch. The oligosaccharides and starches within fiber sources are resistant to hydrolysis of the small intestine and contribute to the "gut-fill" associated with feeding a high-fiber diet (Jarrett et al., 2018). However, consideration must be taken with including fibers as they may have anti-nutritive properties, such as a reduction in the dietary energy and protein (Noblet et al., 2001), and a subsequent decrease in amino acid absorption (Blank et al., 2012).

A common issue that gestating sows experience during late gestation is constipation. This may be a result of the intestine becoming less active as a result of coming parturition (Le Cozler et al., 1999), as well as increased water absorption in preparation for the beginning of milk production (Mroz et al., 1995). Constipation can have a negative impact on the sow's body, resulting in a potential increase in bacterial toxins, which could have a negative impact on the udder (Hou et al., 2014). This is consistent with other studies, in which constipated sows showed higher rates of mastitis than unconstipated sows, which demonstrates a direct effect of constipation on udder health (Hou et al., 2014; Persson, 1996). Further research by Oliviero et al. (2009) found that an increase in fiber content reduced the occurrence of constipation around farrowing and early lactation. During the period from five days before to five days after farrowing, sows fed a 7% crude fiber diet had a softer fecal score compared to sows fed a 3% crude fiber diet (Farmer, 2015).

Another dietary ingredient that has been used in swine diets for constipation alleviation is the addition of magnesium sulfate. The laxative effect of $MgSO_4$ has been studied, and its dietary inclusion has been shown to reduce the incidence of constipation in sows by increasing fecal moisture content (Young et al., 1982; Hou et al., 2014; Zang et

al., 2014). More analyses of essential oil products may need to be performed, but if they contain levels of MgSO$_4$, it may have a laxative effect on sow fecal DM during lactation.

2.13   Conclusion

A sow's body undergoes a wide array of changes in a short time period. Being able to provide her a diet that combats potential issues known to occur in lactation would be ideal. Selecting for prolificacy has resulted in some of the best numbers for the industry in terms of total number born alive and the number of pigs weaned/sow/year. Creating and implementing management programs within the production system that allow us to help the sows and piglets reach their maximum potential will have lasting benefits as reproductive performance is one of the key drivers that influences profits. However, dietary additions such as antibiotic growth promoters have been banned, so finding an alternative feed additive than can positively impact the sow and influence her piglets' growth and efficiency is necessary.

With the variation in piglet BW, gain, and successive weaning weight of sows reared in commercial settings, with the same diet formulation and environment influences, the next logical conclusion is to study the sow herself and determine if there are differences in milk production among individual teats, and how that influences piglet performance prior to weaning. The first step in determining this is to calculate how, if at all, milk yield varies along the udder line, and compare that to the litter performance. If the nutrient composition varies along the sow's udder line, then considerations will have to be taken into account when determining a method for calculating milk yield, as the current methods consider all teats to be equal in terms of production and yield.

Therefore, the objective of the studies herein is to evaluate the effect of supplementation of essential oils on the performance of sow reproduction, milk yield, and piglet pre-weaning growth and development, as well as gain a better biological understanding of piglet nursing habits, and its impact on a piglet's gain.

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Chapter 3. Effect of piglet nursing location along the sow udder line on piglet gain and subsequent weaning weight

3.1 Introduction

One of the major issues that the swine industry is facing today is varied piglet weaning weight within a litter. Individual piglet birthweight is negatively correlated with litter size (Roehe, 1999), and lower birthweight piglets experience lower weight gain and survivability (Gondret et al., 2005). Research by Cabrera et al. (2010) identified a linear relationship ($P < 0.05$) between weaning weight and average daily gain (ADG) in the post-nursery period. Piglets that are lighter at weaning reared under a typical management system may achieve compensatory growth rates during the grow-finish periods but take longer to reach market weight than their heavier counterparts (Mahan and Lepine, 1991). An important aspect of lactation management is understanding the impact that litter size has on overall piglet gain.

Piglet milk intake also influences its overall gain before weaning. The variation in weaning weight is believed to be a result of differences in milk production by each mammary gland (Fraser and Jones, 1975; Fraser et al., 1979). It has been suggested that anterior mammary glands may be larger or produce more milk (Donald, 1937). However, this is in contrast to work done by Hartman et al. (1962) and Pond et al. (1962) that found that there is no difference in milk production among teat glands.

The primary objective of this study was to evaluate whether the piglet nursing location impacts its weaning weight. A secondary objective was to determine how piglet birthweight impacts nursing location. Information about this area of behavior and physiology is limited and increasing the knowledge in this area may result in improved lactation management.

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3.2 Experimental Procedures

This experiment was carried out in environmentally controlled rooms at the University of Kentucky Swine Research Center. The experiment was conducted under protocols approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

3.2.1   Animals and sample collection

A total of 110 sows (York x Landrace) were selected to participate in the study. Over the course of 1 year, all litters that were farrowed were utilized as a part of the observation process. A total of 1,078 individual piglets were initially observed. Piglets were weighed at processing, which occurred within 24 hours of farrowing, and then again at castration and weaning. Nursing observations were recorded at three time points, typically within the same day to verify each piglet's nursing location during the lactation period. Before each observation period, piglets received a number on their back to facilitate data collection. Numbers for each pig were randomly assigned. After being numbered, the entire litter was returned to the sow. During each nursing observation, the teat each piglet nursed and the piglet number was recorded. A nursing bout began when a sow laid down, exposed her stomach and underline, and piglets approached and were attempting to nurse a teat; it was considered to end when the sow rolled over onto her stomach, all piglets moved away from her, or the sow stood up. If a piglet started nursing one teat and then switched to another one, the piglet was only assigned to the teat with which it spent the majority of the nursing period. Teat pairs were labeled from anterior to posterior (1-7). Each pair contained an observation from the two teats included in the pair. Teats that had more than one piglet nursing throughout the observation period were removed from the

analysis. Piglets from litters with a total number at weaning of fewer than six piglets were not utilized.

3.1.2 Statistical Analysis

All data were analyzed by ANOVA with the individual piglet as the experimental unit. The dependent variables evaluated were as follows: birth weight (BW), weaning weight (WW), and weight gain (kg) between days 1 and weaning (WW-BW). The effect of teat location on piglet growth rates was analyzed using the PROC GLM procedure in SAS 9.4 (SAS Institute, Cary, N.C.). The statistical model used litter size as a covariate. Separate analyses for lactation gain (WW-BW) (kg), as well as individual piglet birthweight in comparison to the selected nursing location, was also analyzed in SAS. The model for the analysis of the data was:

$Y_{ij} = k + \alpha_i + e_{i;}$

In this equation, the parameters represent:

k = a constant

α_i = the location effect

e_i = error term of the model

Statistical significance was set at $P < 0.05$, with tendencies for significance at $P < 0.10$.

3.2 Results

3.2.1 Effect of Teat Location on Piglet Weaning Weight

The effect of piglet nursing location on its subsequent weaning weight are shown in Table 3.1 and Figure 3.1. The teat pair that the piglet nurses during lactation does have

a significant effect (P < 0.0001) and there was a linear and quadratic effect on location (L; P < 0.0001); Q; P = 0.0006). There was a significant difference in weaning weight along the udder line of the sow when weaning weight of the piglets were analyzed. Heavier piglets were weaned from the more anterior teats (teat pairs 1-4), although there were no statistical differences between them. Interestingly, the numerically heaviest piglets were not at the most anterior teats (teat pair 1). The heaviest piglets were weaned from teat pair 4 (6.129 kg), and there was a gradual decrease for the piglets nursing the posterior teats, with the lowest weaning weight pigs located at teat pair 7. Litter size at weaning was added as a covariate to the statistical model to account for nursing competition that larger litters may experience. The results are listed in Table 3.2 and Figure 3.2. Litter size does have a significant effect on piglet weaning weight (P < 0.0001) and there was both a linear and quadratic effect on location (L; P < 0.0001; Q; P = 0.0009).

Table 3.1. Average weaning weight (W.W.) in relation to teat pair location

Location[1,3]	W.W. (kg)[2]	S.E.	n
1	5.959^{ab}	0.126	192
2	5.915^{ab}	0.126	185
3	5.996^{ab}	0.127	175
4	6.129^{a}	0.128	168
5	5.746^{b}	0.129	153
6	5.371^{c}	0.138	115
7	5.131^{c}	0.153	90

$^{a-c}$Means within a column without a common superscript differ ($P < 0.05$).
[1]Teat pair location numbered anterior to posterior.
[2]Location effect on weaning weight (P < 0.0001).
[3]Linear and quadratic effect on location (L; P < 0.0001; Q; P = 0.0006).

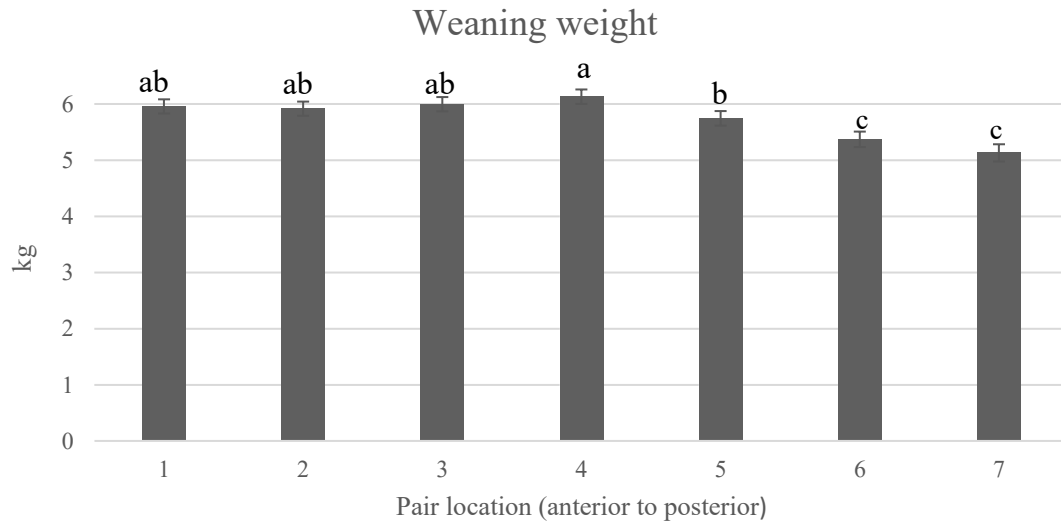Figure 3.1. Average weaning weight in relation to teat pair location

Table 3.2. Average weaning weight (W.W.) using litter size as a covariate in relation to teat pair location

| Location[1,3]	W.W. (kg)[2]	S.E.	n
1	5.946[ab]	0.125	192
2	5.906[ab]	0.125	185
3	5.985[ab]	0.126	175
4	6.121[a]	0.127	168
5	5.745[bc]	0.128	153
6	5.387[cd]	0.137	115
7	5.171[d]	0.155	90

[a-d]Means within a column without a common superscript differ ($P < 0.05$).
[1]Teat pair location numbered anterior to posterior.
[2]Location effect on weaning weight ($P < 0.0001$).
[3]Linear and quadratic effect on location (L; $P < 0.0001$; Q; $P = 0.0009$).

Figure 3.2. Average weaning weight using litter size as a covariate in relation to teat pair location
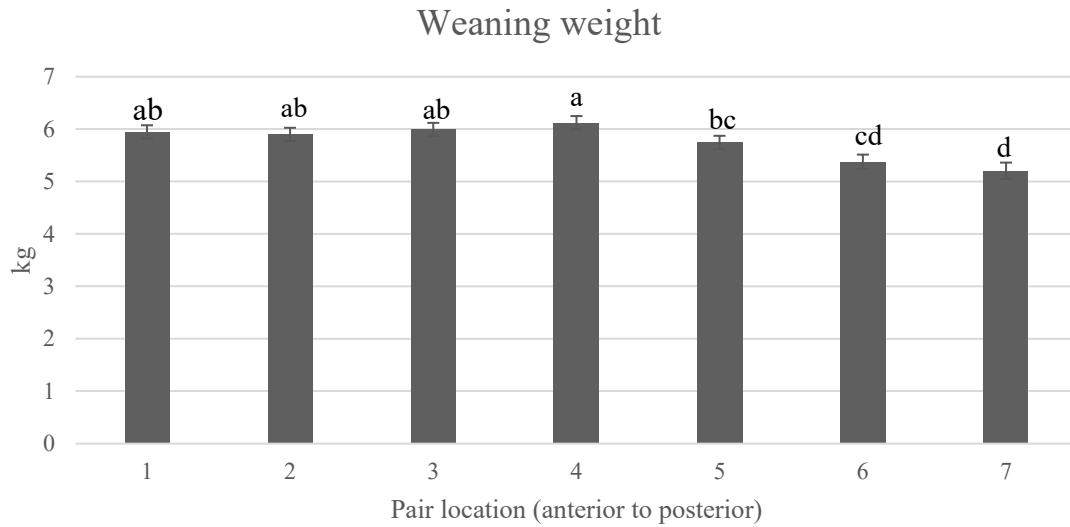
3.2.2. *Effect of Teat Location on Piglet Weight Gain*

To verify that piglet weaning weight differences were impacted by the nursing location, further analysis was done of the actual weight gain of the individual piglet. The goal of this analysis was to determine if piglet gain during the lactation period is still impacted by teat location, or if BW is a contributing factor. Like the results for piglet weaning weight, teat pairs 1-4 had the highest lactation gain ($P < 0.0001$) (Table 3.3 and Figure 3.3). The heaviest piglets obtained the most gain during lactation from teat pair 4. Teat pairs 5-7 had a decrease in lactation gain, with teat pair 7 providing the smallest gain. There was a linear and quadratic effect on location (L; $P < 0.0001$; Q; $P = 0.0033$). Litter size did impact overall lactation gain ($P < 0.0001$), but location no longer impacts gain ($P = 0.57$). These results are shown in Table 3.4 and Figure 3.4. While weaning weight along the udder line suggested that there may be a difference in either production or nutrient

composition along the udder line, when actual piglet weight gain is assessed, the first four pairs of teats seem to produce relatively similar outcomes, as the values for teat pairs 1-4 do not differ significantly.

Table 3.3 Average piglet gain in relation to teat pair location[4]

Location[1,3]	Gain (kg)[2]	S.E.	n
1	4.418[a]	0.114	192
2	4.421[a]	0.115	185
3	4.470[a]	0.116	175
4	4.495[a]	0.116	168
5	4.175[bc]	0.118	153
6	3.898[bc]	0.125	115
7	3.699[c]	0.138	90

[a-d]Means within a column without a common superscript differ ($P < 0.05$).
[1]Teat pair location numbered anterior to posterior.
[2]Location effect on piglet gain ($P < 0.0001$).
[3]Linear and quadratic effect on location (L; $P < 0.0001$; Q; $P = 0.0033$).
[4]Piglet gain = piglet weaning weight-birthweight.

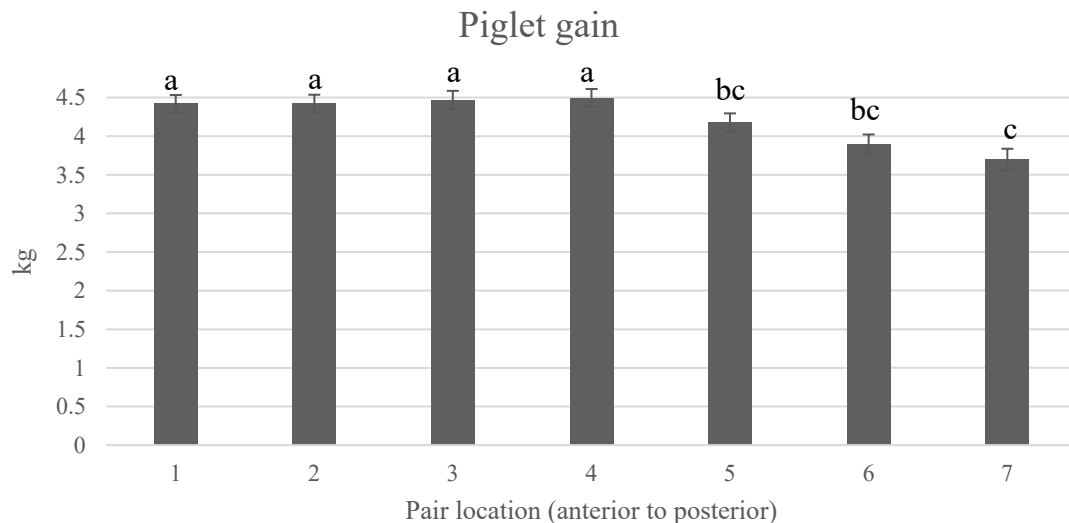Figure 3.3. Average piglet gain in relation to teat pair location

Table 3.4. Average piglet gain in relation to teat pair location using litter size as a covariate[4]

Location[1,4]	Gain (kg)[2,3]	S.E.	n
1	4.406[a]	0.113	192
2	4.404[a]	0.114	185
3	4.465[a]	0.115	175
4	4.489[a]	0.115	168
5	4.175[ab]	0.117	153
6	3.909[bc]	0.125	115
7	3.756[c]	0.141	90

[a-c]Means within a column without a common superscript differ ($P < 0.05$).
[1]Teat pair location numbered anterior to posterior.
[2]Location effect on piglet gain ($P < 0.0001$).
[3]Linear and quadratic effect on location (L; $P < 0.0001$; Q; $P = 0.0051$).
[4]Piglet gain = piglet weaning weight – birthweight.

Figure 3.4 Average piglet gain in relation to teat pair location using litter size as a covariate
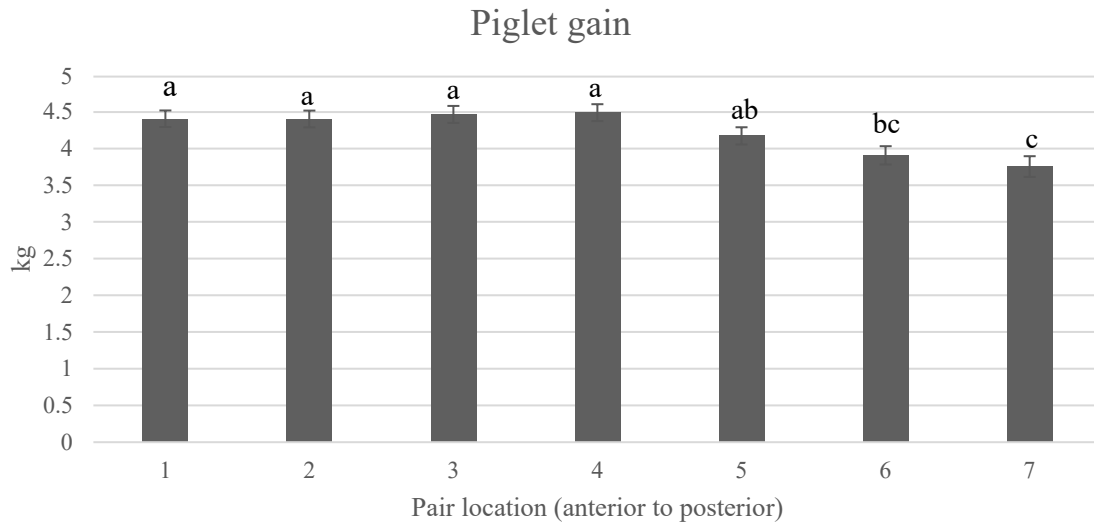
3.2.2. *Effect of Piglet Birthweight on Nursing Location*

Piglets that initially select the first or second pair of teats have been reported to have the heaviest birth weights in the litter (Lannom et al., 2018). In the previous analyses within this experiment, the first four anterior teat pairs typically yielded the highest results in both

weaning weight and piglet gain. The BW of piglets did not impact their teat preference (P

= 0.16) and there was a linear tendency and quadratic effect on location (L; P = 0.09; Q;P

= 0.05). These results are shown in Table 3.5 and Figure 3.5. Typically, litter size impacts

birthweight, with larger litters producing smaller pigs at birth (Quiniou et. al., 2002;

Beaulieu et al., 2010). Litter size did not significantly impact teat selection based off piglet

birthweight (P = 0.29), and there was no longer a linear tendency (P = 0.11), but there was

still a quadratic effect on location (P = 0.05). These results are shown in Table 3.6 and

Figure 3.6, respectively.

Table 3.5. Average piglet birthweight (B.W.) in relation to teat pair location

Location[1,3]	B.W. (kg)[2]	S.E.	n
1	1.542	0.031	192
2	1.506	0.032	185
3	1.531	0.032	175
4	1.581	0.032	168
5	1.537	0.035	153
6	1.498	0.034	115
7	1.443	0.038	90

[1]Teat pair location numbered anterior to posterior.
[2]Location effect on birthweight (P < 0.0001).
[3]Quadratic effect on location (P = 0.05).

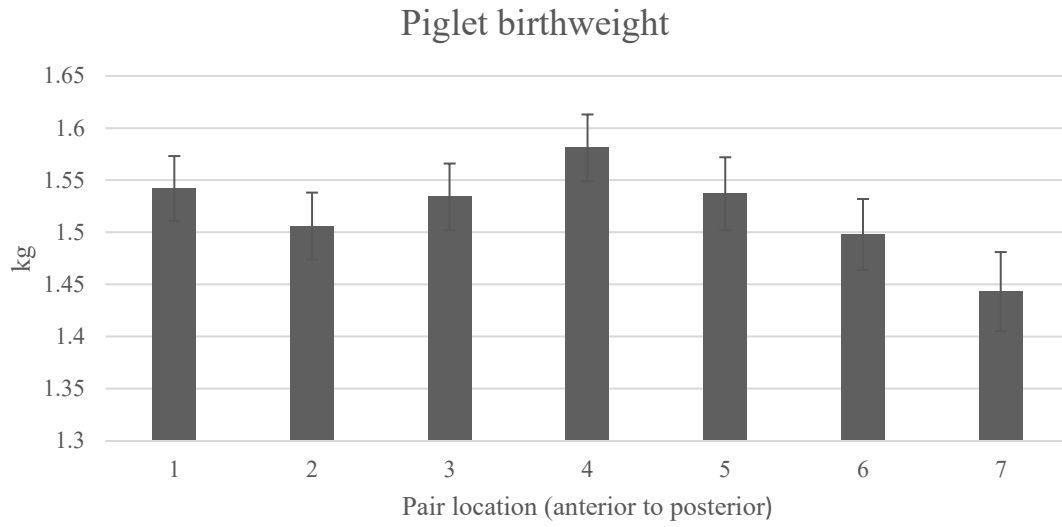Figure 3.5. Average piglet birthweight in relation to teat pair location

Table 3.6. Average piglet birthweight (B.W.) in relation to teat pair location using litter size as a covariate

Location[1,3]	B.W. (kg)[2]	S.E.	n
1	1.541	0.031	192
2	1.507	0.031	185
3	1.530	0.032	175
4	1.581	0.032	168
5	1.537	0.032	153
6	1.499	0.034	115
7	1.446	0.038	90

[1]Teat pair location numbered anterior to posterior.
[2]Location effect on birth weight ($P < 0.0001$).
[3]Quadratic effect on location ($P = 0.05$).

Figure 3.6. Average piglet birthweight in relation to teat pair location using litter size as a covariate

Piglet birthweight
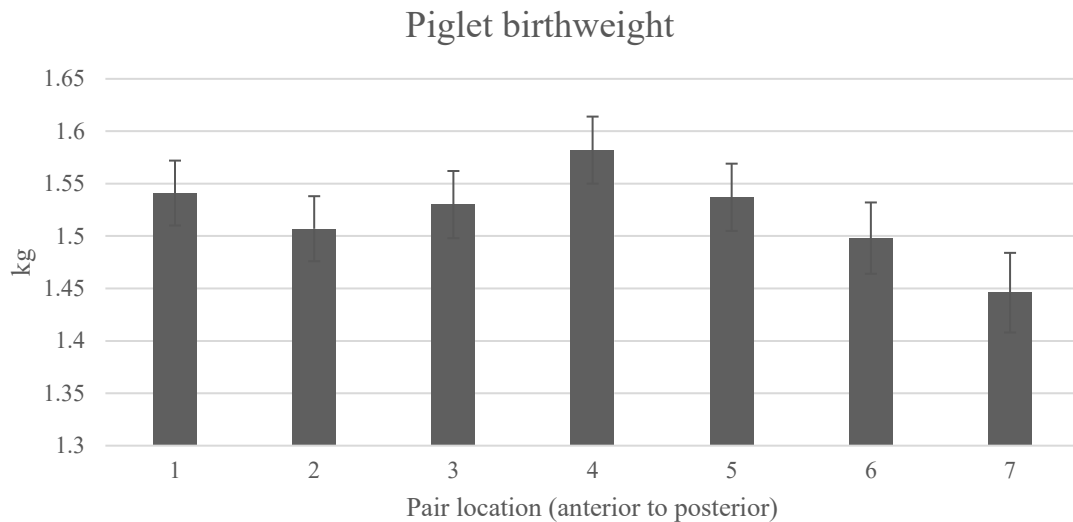
3.3 **Discussion**

One of the main objectives of this study was to gain an understanding of the nursing behavior in piglets and correlate their recorded nursing location with subsequent growth parameters and obtain a better biological understanding of how, or if, milk production may change along the udder. While nursing behavior has been studied previously, at the beginning of this experiment, very little was known about how production may vary. Based on the present results, the first four pairs of anterior teats produce the heaviest weaning weights, but when birthweight of the piglet is accounted for, the output and nutritional value may be similar, as the average gain was not statistically different across those four pairs. In contrast, the piglets nursing the posterior pairs five through seven had overall reduced growth characteristics. It is hypothesized that lighter birthweight piglets get pushed to the more posterior teats, and the results would agree with that, as the resulting weights were lower in all aspects.

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Lannom et al. (2018) reported that individual nutrient components of both milk and colostrum were statistically different among teat pairs. This experiment did not measure the individual components of teat pairs, but from the results, it does appear that there are biological differences between teat pairs that results in a decreased gain of piglets along the udder line. This would agree with work done by Skok et al. (2007), which found that piglets nursing from teats considered to be anterior or middle pairs did not consume a statistical difference in milk during nursing to affect weight gain, but in comparison to the posterior teats found a significant difference in the quantity of milk consumed (P < 0.05). This experiment does give a better biological understanding of the sow udder line. If the components and output along the udder line were nutritionally similar, then weight gain would be more consistent overall. From a management perspective, this has some interesting implications that will need to be considered. If the nutritional composition and/or output decreases significantly the more posterior the piglets nurse, then management practices, such as cross-fostering, providing creep feed to the litter, or nutritional considerations such as altering the sow diet to impact milk yield or nutrient components, may need to be taken into account in an effort to combat the nutritional detriment that potentially smaller, lighter piglets will be experiencing if they are nursing from a posterior teat pair.

3.4   Conclusion

The present study shows that piglet gain and subsequent weaning weight is ultimately impacted by their preferred nursing location along the udder line. This provides some biological insight in understanding the differences in either milk nutrient composition,

yield, or a combination of both that the sow produces. The next logical step may be to gather samples from every teat during lactation to obtain a better understanding of how or if composition changes from parturition to weaning. The sample collection should also occur at numerous time points in order to gain an understanding of how composition may change over the lactation period. Piglet gain should also be measured using previously validated methods (weigh-suckle-weigh, D_2O). This would provide a better understanding about milk yield along the udder line, and potential differences between teat pairs. If nutritional composition and or yield are not consistent along the udder line, then equations used to calculate milk yield may need to be re-evaluated. As the modern genetic sow lines continue to select for prolificacy, steps will need to be implemented to provide large litters of piglets with the opportunity for teat access that provides the best opportunity for piglet growth.

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Chapter 4. The impact of essential oil supplementation on sow fecal dry matter, colostrum and milk composition, and piglet weaning weight

4.1 Introduction

The transition from gestation to lactation can significantly impact a sow's body. During late pregnancy, a common practice is to feed the sow a reduced feed amount but increase the concentration of energy available within the diet. Concentrated diets typically contain a more limited amount of fiber. This is due in part to providing the sow with enough energy for upcoming milk production (Einarsson and Rojkittikhun, 1993). Before parturition, the sow's intestinal activity decreases (Oliviero et al., 2009) and water absorption within the small intestine increases in preparation for the upcoming milk production (Mroz et al., 1995). These changes in intestinal activity can result in subsequent constipation post-farrowing. Constipation can cause discomfort to the sow, is associated with udder infections during late lactation (Hou et al., 2014; Martineau et al., 1992; Persson, 1996), and may also result in a decreased feed intake. Constipation can also influence the release and absorption of bacterial endotoxins, which can lead to the development of post-partum dysgalactia in sows (Tabeling et al., 2003). Different dietary additions have been utilized in the past to alleviate the potential for constipation. One common addition to the diet used to offset constipation is magnesium sulfate. It has been successfully used as a laxative to help prevent constipation (Young, 1982). Supplementation of sows with additional fiber (Darroch et al., 2008; Weber et al., 2014) has also successfully softened stool texture, resulting in a decrease in constipation.

In recent years, essential oils have received more interest as a dietary addition that may improve growth rate and feed efficiency (Zeng et al., 2015; Cromwell, 2002). They have been shown to have antimicrobial and anti-inflammatory properties and enhance

digestibility (Omonijo et al., 2018). Supplementation of oregano essential oils (OEO) may increase voluntary feed intake in lactating sows (Allan and Bilkei, 2005), and may influence overall milk yield. Khajarern and Khajarern (2003), found that supplementing lactating sows with OEO at a rate of 0.025% in the diets produced a higher daily milk yield of 10.44 kg (OEO) vs. 9.53 kg. The objective of this study was to determine the effects of a liquid essential oil (EO) product (tradename Absorbezz®) available in health food stores on sow fecal dry matter, colostrum and milk component composition, immunoglobulin levels and overall piglet weaning weight.

4.2 Experimental procedures

This experiment was carried out in environmentally controlled rooms at the University of Kentucky Swine Research Center. The experiment was conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

4.2.1 Experiment 1

A total of 62 sows (Yorkshire x Landrace or Yorkshire) from two farrowing groups with an average parity of 3.03 ± 1.98, were assigned to 2 dietary treatments: 1) control diet that met NRC [2012] nutrient requirements and 2) the control diet with an essential oil product top-dressed onto the daily feed ration [10 mL/d]. The essential oil product (Absorbezz®O; Absorbezz LLC; Ft. Lauderdale, FL) was the product used in this experiment. Absorbezz® contains complex ionic minerals, 72 trace minerals, calcium carbonate, and mastic gum, derived from the Chois Mastiha tree. Sows were allotted to treatment based on parity, breed, and breeding weight. Sows were housed in individual gestation stalls (0.57 x 2.13 m^2), with the rear 0.66 m slatted with concrete slats. Individual

floor feeding at a level of 1.8 kg/d was maintained throughout gestation and water was available on an ad libitum basis from nipple waterers. The experiment began following the movement of sows into the farrowing rooms.

On approximately D 108 of gestation, sows were moved to a temperature-regulated farrowing facility and placed in farrowing stalls (1.52 x 2.13 m^2) with plastic-coated welded wire flooring, heating lamps and nipple waterer for piglets, and a drinking nipple and feed trough for sows. Sows were provided with 3.2 kg of lactation diet on the day of farrowing, and then gradually increased until daily feed intake reached at least 6.4 kg; thereafter sows were allowed to consume their diets on an ad libitum basis for the remainder of lactation. On the day of weaning, approximately D 21 of lactation, sows were returned to the breeding facility to begin detection of estrous and rebreeding. Gestation room temperature and farrowing/lactation room temperature and humidity were recorded daily.

4.2.2 Experimental Diets

The diets consumed by the animals were formulated to meet or exceed NRC (2012) nutrient requirement estimates for gestating and lactating sows (Table 4.1). Minerals and vitamins were added to meet or exceed NRC (2012).

Table 4.1. Percentage composition of the basal diets for sows (as-fed basis)

Ingredient	Gestation %	Lactation %
Corn	73.03	67.00
Dehulled soybean meal	19.00	25.60
Alfalfa meal	2.00	2.50
L-Lysine	0.00	0.96
Dicalcium Phosphate	1.56	1.21
Limestone	1.00	0.89
Choice white grease	2.00	1.00
Choline chloride - 50%	0.10	0.10
Salt	0.50	0.50
Clay product AB-20	0.50	0.50
Chromax[1]	0.05	0.05
Trace-mineral mix[2]	0.15	0.15
Vitamin mix[3]	0.05	0.05
Santoquin[4]	0.02	0.02
Calculated nutrient composition		
ME, kcal/g	3,301	3,240
CP, %	15.47	18.19
Lysine, %	0.81	0.97
Calcium, %	0.83	0.75
Phosphorus, %	0.62	0.60

[1] Chromax (Prince Agri Products, Inc., Quincy, IL) provided 200 ppb Cr as chromium tripicolinate.
[2]Premix (Prince Agri-Products, Quincy, IL) provided 7.50 ppm Ca ($CaCO_3$), 75 ppm Mn (MnO), 165 ppm Zn ($ZnSO_4$), 165 ppm Fe ($FeSO_4$), 27 ppm Cu ($CuSO_4$), 1.05 ppm I ($Ca(IO_3)_2$), and 0.15 ppm Se ($Na_2(SeO_4)$) in the final diet.
[3]Premix (Provimi North American, Brookville, OH) provided 5,306.50 IU of vitamin A, 1,327.50 IU vitamin D_3, 35.32 IU vitamin E, 3.93 IU vitamin K, 1.30 mg menadione, 0.015 mg vitamin B_{12}, 0.13 mg biotin, 0.09 mg folic acid, 23.50 mg niacin, 11.82 mg d-pantothenic acid, 2.36 mg pyroxidine, and 0.65 mg thiamine in the final diet.
[4]Santoquin (Monsanto, St. Louis, MO) provided 130 mg/kg ethoxyquin to the diet.

4.2.3 Data and Sample Collection

Sow feed consumption during lactation was recorded daily. Sow weights were obtained at breeding, pre-farrowing (gestation D 108-110), within 24 h post-farrowing, and at weaning. The number of pigs born alive and dead, as well as the birth weight of each individual pig, was recorded within 24 h of farrowing. In addition, piglets received ear-

notches, clipping of needle teeth, and an injection of 150 mg Fe as Fe dextran on the same day. Male piglets were castrated between D 6-8 of age. Creep feed was not offered, but access to the sow's feed was not restricted. At weaning, individual piglet weaning weights were recorded.

Fecal samples from all sows were retrieved by grab collection in late gestation and lactation. The collection time points were between D 108-110 of gestation, D 4-6, and D 14-17 of lactation. Samples were placed in containers, weighed, and stored at -20 °C until further analysis.

Milk samples were hand expressed from each sow during D 14-17 of lactation. Each sow received an injection of 1 mL oxytocin (OXOJECT, Henry Schein Animal Health, Dublin, Ohio) in an ear vein. Milk samples were immediately placed on ice, aliquoted into containers, and stored at -20 °C until analyzed for the components of fat, protein, lactose, total solids, and solids non-fat.

Milk yield of a 21-D lactation period was predicted by a Bayesian hierarchical model based on litter size and litter weight gain (Hansen et al., 2012). Since the predicted milk yield was in the gravimetric unit (kg) it was converted to the volumetric unit (L) by dividing the predicted yield by the density of each milk sample.

4.2.3.1 Laboratory Analysis

Milk samples were stored as raw milk at -20 °C before compositional analysis. The raw milk samples were thawed before delivery to the milk laboratory of the Division of Regulatory Services, University of Kentucky to analyze for fat, protein, lactose, total solids, and solids non-fat. The gross energy content of the complete milk was calculated

from the concentration of protein, fat, and lactose, which contribute 16.4 kJ/g, 38.9 kJ/g, and 23.8 kJ/g, respectively (Ramanau et al., 2004).

Fecal samples were thawed at room temperature overnight and then dried in a forced-air drying oven at 55°C for 1 week. Samples were checked and weighed daily until the weight change was less than 0.03 g. The dried fecal samples were air equilibrated, weighed, and ground through a 1 mm screen using a Wiley Laboratory Mill (model 3; Arthur H. Thomas Co., Philadelphia, PA) for chemical analysis.

Fecal samples were analyzed for dry matter (DM). Dry matter was assessed according to AOAC (1990) methods, which involved further overnight drying (105°C) of the dried samples in a convection oven (Precision Scientific Co., Chicago, IL).

4.2.3.2 Statistical Analysis

All data were analyzed by ANOVA in a completely randomized design with sow as the experimental unit. Analysis of variance was performed using the GLM procedure of SAS v. 9.4 (SAS Inst. Inc., Cary, NC) with lactation length used a covariate for reproductive performance. When testing for interactions, these sows were considered group 1 and group 2. Piglet data represented observed nursing location on a teat, and each teat pair represents the average weaning weight (WW), lactation gain (weaning weight-birthweight), as well as birthweight (BW), as explained in Chapter 2. The model for the analysis of the data was:

$$YY = kk + GG_{ii} + OO_{jj} + GGOO_{iijj} + EE_{kk(iijj)}$$

In this equation, the parameters represent:

k = a constant

G_i = the group effect (across groups of the sows fed)

O_j = the essential oil treatment effect

GO_ij = the interaction of group and treatment effect

$E_{k(ij)}$ = error term for the model

Piglet data were analyzed in the same way as Chapter 3, with the addition of G_i for group effect, and an additional interaction in $\alpha_i O_j$, which tested for an interaction between nursing location and treatment effect.

Experiment 2

4.3.1   Animals and treatments

A total of 32 sows (Yorkshire or Landrace x Large White) with an average parity of 2.22 ± 2.20 were assigned to 2 dietary treatments: 1) control diet that met NRC [2012] nutrient requirements, and 2) the control diet with an essential oil product [0.685% for gestation, 0.40% and 0.20% for lactation Phases I and II diets]. The Phase I diet was formulated to meet requirements for lactating sows with an ADFI up to 7 kg, with Phase II diets being formulated for sows with an ADFI above 7 kg. The essential oil product (Absorbezz®O; Absorbezz LLC; Ft. Lauderdale, FL) was the product used again in this experiment.

Sows were allotted to treatment based on parity, breed, and breeding weight and were housed, fed, and handled as in Experiment 1. The experiment began approximately 27 days before the expected farrowing date.

4.3.2 Experimental Diets

The diets consumed by the animals were formulated to meet or exceed NRC (2012) nutrient requirement estimates for gestating and lactating sows (Table 4.2). Minerals and vitamins were added to meet or exceed NRC (2012).

Table 4.2. Percentage composition of the basal diet for sows (as-fed basis)

Ingredient	Gestation %	Lactation %
Corn	76.50	69.57
Dehulled soybean meal	19.00	27.00
Corn starch	0.75	0.20
L-Lysine	0.06	0.04
Dicalcium Phosphate	1.55	1.60
Limestone	1.00	0.90
Chromax[1]	0.05	0.05
Choline chloride - 60%	0.10	0.10
Salt	0.50	0.50
Copper sulfate pentahydrate	0.02	0.02
Trace mineral mix[2]	0.10	0.10
Vitamin mix[3]	0.05	0.05
Santoquin[4]	0.02	0.02
Titanium dioxide	0.30	0.30
Calculated nutrient composition		
ME, kcal/g	3,253	3,240
CP, %	15.42	18.66
Lysine, %	0.69	1.00
Calcium, %	0.83	0.84
Phosphorus, %	0.62	0.67

[1] Chromax (Prince Agri Products, Inc., Quincy, IL) provided 200 ppb Cr as chromium tripicolinate.
[2] Premix (Prince Agri-Products, Quincy, IL) provided 7.50 ppm Ca ($CaCO_3$), 75 ppm Mn (MnO), 165 ppm Zn ($ZnSO_4$), 165 ppm Fe ($FeSO_4$), 27 ppm Cu ($CuSO_4$), 1.05 ppm I ($Ca(IO_3)_2$), and 0.15 ppm Se ($Na_2(SeO_4)$).
[3] Premix (Provimi North American, Brookville, OH) provided 5,306.50 IU of vitamin A, 1,327.50 IU vitamin D_3, 35.32 IU vitamin E, 3.93 IU vitamin K, 1.30 mg menadione, 0.015 mg vitamin B_{12}, 0.13 mg biotin, 0.09 mg folic acid, 23.50 mg niacin, 11.82 mg d-pantothenic acid, 2.36 mg pyroxidine, and 0.65 mg thiamine.
[4] Santoquin (Monsanto, St. Louis, MO) provided 130 mg/kg ethoxyquin to the diet.

4.3.3 Data and Sample Collection

Fecal sample collection and storage was the same as described in Experiment 1. Colostrum and milk samples were collected from each sow during the lactation period. Colostrum was collected within 8 hr. of the onset of parturition. Sows received an intramuscular injection of 1 mL of oxytocin prior to collection. Milk sample collection was the same as described in Experiment 1. Colostrum and milk samples were immediately placed on ice, aliquoted into containers, and stored at -20 °C until analyzed for components. An aliquot of both colostrum and milk from each sow were centrifuged at 9,950 x g at 4 °C for 20 and 10 minutes respectively, to separate the fat from the skim layer. The fat layer was removed and discarded and the skimmed colostrum and milk samples were then centrifuged at 39,800 x g at 4 °C for 45 and 20 minutes, respectively, to separate the whey fractions. The whey fractions of colostrum and milk samples were stored at -20 °C until further analysis of the immunological components of IgA, IgG, and IgM. Milk yield was calculated in the same way as Experiment 1. The data collected from this sow group was considered group 3.

4.3.4.1. Laboratory Analysis

Colostrum and milk samples were stored as raw milk at -20 °C before compositional analysis. They were analyzed for the same components as described in Experiment 1. Fecal samples were prepared the same as described in Experiment 1.

Feed and fecal samples were analyzed for TiO_2 with the intent of determining digestibility dry matter (DM), gross energy (GE), ether extract (EE), and nitrogen. The titanium dioxide determination method validated by Fowler (2018) was utilized for both

feed and fecal samples. The detailed procedure and methods validation is described in Appendix II.

Total IgA, total IgG, and total IgM were measured in all colostrum and milk whey samples by enzyme-linked immunosorbent assay (ELISA) test (pig IgA/IgG/IgM ELISA quantitation kit, Bethyl Laboratories Inc., Montgomery, TX) following the manufacturer's protocol. Detailed analysis procedure is described in Appendix I.

4.3 Results

Essential oil supplementation did not affect sow fecal DM at any point during the experiment. There was nearly no detectable difference between the two treatment groups at any time point (Table 4.3).

Table 4.3. Effect of essential oil (EO) supplementation on fecal DM (%) from late gestation through weaning

| Timepoint[1] | Treatment | | | |
	CON	EO	SEM	P-value
n	41	44		
Late Gestation	35.78	35.69	1.73	0.94
Early Lactation	36.91	36.75	0.89	0.89
Late Lactation	28.35	28.30	0.48	0.89

[1]Essential oils were top-dressed in Experiment 1 and incorporated into the diet in Experiment 2.

4.3.1 Colostrum and Milk Composition

The colostrum composition (Table 4.4) was not significantly affected by the supplementation of essential oils (P > 0.10). The composition of milk (Table 4.5) was significantly different between the CON and the EO groups for the components of lactose (P = 0.04), with the EO treatment group producing higher levels (5.97% vs. 5.84%). There

were also tendencies to decrease solids non-fat (P = 0.07) and gross energy (P = 0.08) for the sows supplemented with the Absorbezz®.

The amount of milk yield per litter and piglet was not affected by the addition of the essential oil (P > 0.25). The overall predicted milk yield was higher for the essential oil supplementation group (172.55 kg vs. 164.54 kg) (Table 4.7).

Table 4.4. Effect of essential oil (EO) supplementation on colostrum composition [1]

	Treatment			
Component	CON	EO	SEM	P-value
n	16	16		
Fat (%)	5.13	5.08	0.43	0.94
Protein (%)	15.75	14.23	0.74	0.16
Lactose (%)	2.84	3.09	0.14	0.21
Gross energy[2] (MJ/kg)	5.26	5.04	0.21	0.50
Total solids (%)	27.28	26.00	0.45	0.32
Solids non-fat (%)	20.95	19.87	0.65	0.25

[1]Samples were collected from experiment 2.
[2] The gross energy content of the complete milk was calculated from the concentration of protein, fat, and lactose, which contribute 16.4 kJ/g, 38.9 kJ/g, and 23.8 kJ/g, respectively (Ramanau et al., 2004).

Table 4.5. Effect of essential oil (EO) supplementation on milk composition[1]

	Treatment			
Component	CON	EO	SEM	P-value
n	30	32		
Fat	5.94	5.53	0.19	0.89
Protein	4.84	4.66	0.06	0.12
Lactose	5.84	5.97	0.05	0.04
Gross energy[2]	4.45	4.29	0.07	0.08
Total solids	17.66	17.14	0.19	0.11
Solids non-fat	11.00	10.91	0.05	0.07

[1]Samples were collected from group 2 and 3.
[2] The gross energy content of the complete milk was calculated from the concentration of protein, fat, and lactose, which contribute 16.4 kJ/g, 38.9 kJ/g, and 23.8 kJ/g, respectively (Ramanau et al., 2004).

The immunoglobulin levels of colostrum and milk are presented in Table 4.6. There was no significant impact on the immunoglobulin levels in the colostrum samples

(P > 0.30). Similar results were determined in the milk samples, as the differences among treatments were not significant (P > 30.)

Table 4.6. Effect of essential oil (EO) supplementation on colostrum and milk immunoglobulin levels[1]

	Treatment			
Item	CON	EO	SEM	P-value
n	16	16		
Colostrum[2]	16	16		
IgA, mg/mL	0.61	0.60	0.11	0.95
IgG, mg/mL	153.97	176.6	18.19	0.40
IgM, mg/mL	3.05	3.54	0.36	0.35
Milk[2]				
IgA, mg/mL	3.36	3.31	0.51	0.94
IgG, mg/mL	0.33	0.28	0.04	0.31

[1]Samples were collected from experiment 2.
[2]For analytical details, see Appendix I.

There was a significant effect on sow weight change during lactation (P = 0.002) when lactation length was used as a covariate (Table 4.7). The EO group had an overall increase in mean weight loss (13.49 vs. 3.17) compared to the CON group. There was no statistical impact on sow milk production during lactation (P = 0.27). Essential oil supplementation did not have an effect on overall sow ADFI (P > 0.50).

Table 4.7. Effects of essential oil (EO) supplementation on sow lactation performance with lactation length as a covariate

| | Diet | | | |
	CON	EO	SEM	P-values
No. of litters	38	41		
Sow weight, kg				
Late Gestation	258.21	249.47	4.63	0.19
Farrowing	235.22	242.34	4.79	0.29
Weaning	232.05	228.81	5.45	0.37
Sow weight loss, kg				
Lactation	3.17	13.49	2.35	**0.002**
Lactation daily feed intake, kg/d	5.27	5.41	0.16	0.55
Milk production, kg[1]	164.54	172.55	5.07	0.27
Litter size				
Total born	12.18	12.48	0.52	0.68
Live born	10.01	10.28	0.65	0.76
Weaning	9.64	9.68	0.41	0.80
Piglet data, without covariate				
Live birthweight (kg)	1.49	1.57	0.02	0.01
Piglet gain (kg)	4.41	4.45	0.11	0.78
Weaning weight (kg)	5.93	6.03	0.12	0.53
Piglet data, with covariate				
Live birthweight (kg)	1.49	1.56	0.02	0.03
Piglet gain (kg)	4.66	4.40	0.08	0.02
Weaning weight (kg)	6.18	5.95	0.10	0.14

[1] Milk yield of a 21-d lactation period was predicted by a Bayesian hierarchical model based on litter size and litter weight gain (Hansen et al., 2012).

4.3.2 *Piglet Weaning Weight and Growth Performance Without Covariates*

Piglet growth rate was analyzed using the same methods found in Chapter 3. Initially, growth rate was analyzed without any covariates used. Piglet WW was not affected by supplementation of EO to the sows (P = 0.53) (Table 4.8). There was no TRT*Location interaction (P = 0.64). There was a linear effect of teat location for piglet weaning weight (P = 0.03), but there was no quadratic effect on location (P = 0.17). Mean WW can be found in Table 4.7 and Figure 4.1.

Essential oil supplementation did not impact overall piglet lactation gain (P = 0.78). There was a linear effect of teat location (P = 0.03), and like the WW analysis, there was

no quadratic effect on location (P = 0.25). These results are located in Table 4.9 and Figure 4.2.

The supplementation of the essential oils did impact piglet birth weight between the two groups, with piglets from the EO treatment weighing 1.57 kg at birth compared to those in the CON at 1.49 kg (P = 0.01), found in Table 4.10 and Figure 4.3. There was no significant impact of piglet birth weight on nursing location (P = 0.65) (Table 4.10), and there was no linear or quadratic effect on location (L; P = 0.40; Q; P = 0.68).

Table 4.8. Effects of essential oil (EO) supplementation on piglet weaning weight (kg) in relation to teat pair location[2]

| | Treatment | | | |
Location[1]	CON	EO	SEM	n
1	5.93	6.53	0.29	100
2	5.79	6.17	0.29	96
3	5.92	6.22	0.28	98
4	6.47	6.23	0.29	94
5	6.13	6.03	0.29	94
6	5.78	5.44	0.31	76
7	5.47	5.57	0.34	55

[1]Teat pairs were numbered from anterior to posterior along the udder line.
[2]Linear effect of location (P = 0.03).

Figure 4.1. Effects of essential oil (EO) supplementation on piglet weaning weight (kg) in relation to teat pair location
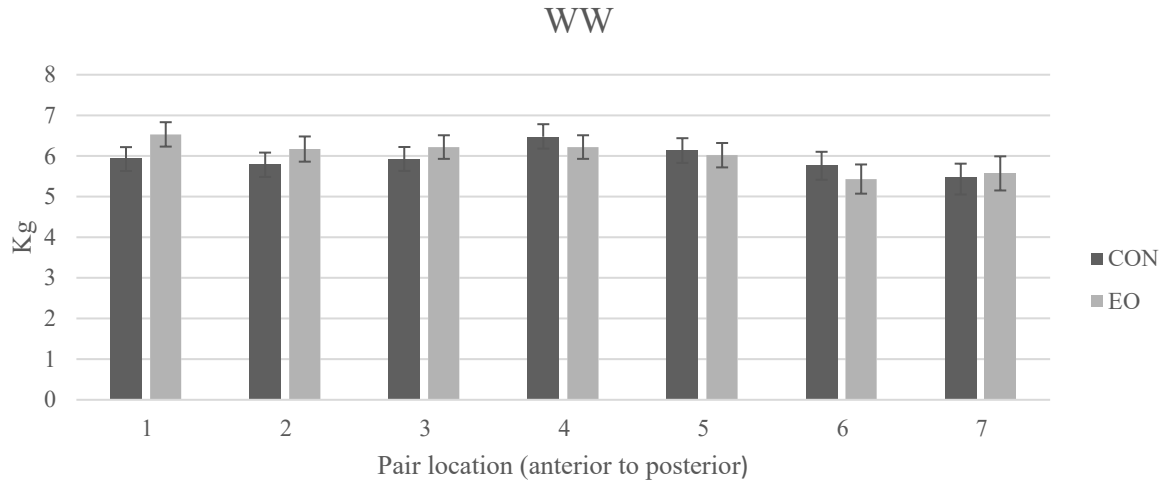

WW

Table 4.9. Effects of essential oil (EO) supplementation on piglet gain (kg) in relation to teat pair location[2]

Location[1]	Treatment		SEM	n
	CON	EO		
1	4.40	4.88	0.27	100
2	4.35	4.63	0.27	96
3	4.45	4.59	0.26	98
4	4.56	4.69	0.26	94
5	4.39	4.39	0.27	94
6	4.03	3.89	0.31	76
7	3.92	4.08	0.34	55

[1]Teat pairs were numbered from anterior to posterior along the udder line.
[2]Linear effect of location (P = 0.03).
[3]Piglet gain = piglet weaning weight – birthweight.

Figure 4.2. Effects of essential oil (EO) supplementation on piglet gain (kg) in relation to teat pair location

Piglet gain

kg — Pair location (anterior to posterior)

CON
EO

Table 4.10. Effects of essential oil (EO) supplementation on piglet birthweight (kg) and selection of nursing location[2]

	Treatment			
Location[1]	CON	EO	SEM	n
1	1.51	1.66	0.06	100
2	1.45	1.54	0.06	96
3	1.45	1.62	0.06	98
4	1.51	1.56	0.06	94
5	1.55	1.61	0.06	94
6	1.53	1.53	0.06	76
7	1.46	1.49	0.07	55

[1]Teat pairs were numbered from anterior to posterior along the udder line.
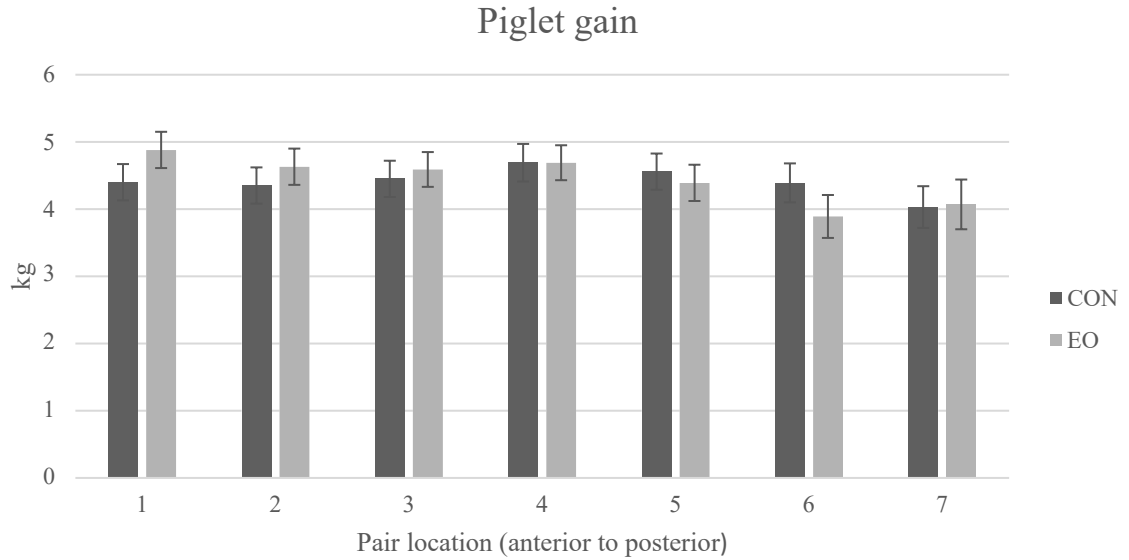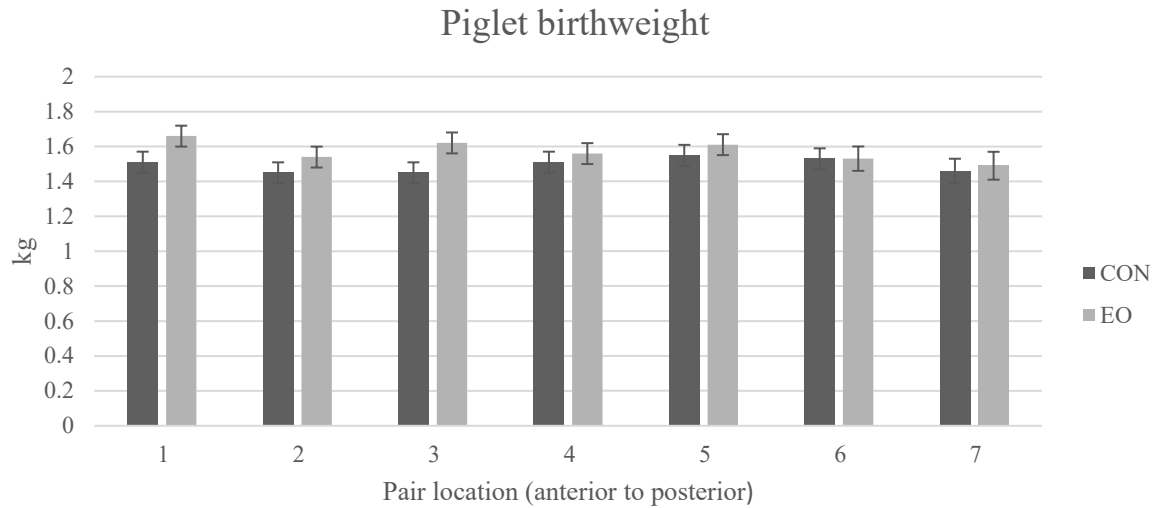[2]Treatment effect on piglet birthweight (P = 0.01).

Figure 4.3. Effect of essential oil (EO) supplementation on piglet birthweight (kg) and selection of nursing location

4.3.3 Piglet Growth Performance with Covariate Analysis

The supplementation of essential oils did not impact piglet weaning weight ($P = 0.15$). Litter size did not impact piglet weaning weight ($P = 0.22$), but piglet nursing location still had a statistically significant effect on weaning weight ($P = 0.008$) found in Table 4.11 and Figure 4.4. As anticipated, lactation length also had a significant effect on piglet weaning weight ($P < 0.0001$). Piglet gain during lactation was significantly impacted by essential oil supplementation ($P = 0.02$), with piglets from CON sows gaining more (4.66 kg) than EO piglets (4.40 kg) (Table 4.7). Litter size did not impact piglet gain ($P = 0.27$), and there continued to be a significant effect from lactation length ($P < 0.0001$). There was a linear effect ($P = 0.01$) of teat location, but there was no TRT*location interaction ($P = 0.32$). There was no Group*TRT interaction ($P = 0.85$), but there was a Group effect ($P < 0.001$). Individual location comparisons between the treatment groups are found in Table 4.12 and Figure 4.5.

PROC GLM in SAS with covariate analysis determined that essential oil supplementation did significantly impact piglet BW, with piglets born from the EO supplementation group weighing 1.56 kg vs. 1.49 in the CON (P = 0.003) (Table 4.7). LS did impact BW significantly (P = 0.05). Individual teat pair comparisons are found in table 4.13 and Figure 4.6. This agrees with previous work (Quiniou et al., 2002) that LS does significantly impact mean piglet BW.

Table 4.11. Effect of essential oil (EO) supplementation on piglet weaning weight (kg) at different locations with litter size and lactation length as covariates [2,3]

| | Treatment | | | |
Location[1]	CON	EO	SEM	n
1	6.10	6.39	0.24	100
2	6.07	6.12	0.24	96
3	6.26	6.27	0.24	98
4	6.70	6.19	0.24	94
5	6.34	5.94	0.24	94
6	6.14	5.43	0.26	76
7	5.67	5.23	0.28	55

[1]Teat pairs were group from anterior to posterior along the udder line.
[2]Location effect (P = 0.02).
[3]Lactation length effect (P < 0.0001).

Figure 4.4. Effect of essential oil (EO) supplementation on piglet weaning weight (kg) at different locations with litter size and lactation length as covariates

Table 4.12. Effect of essential oil (EO) supplementation on piglet gain (kg) at different locations with litter size and lactation length as covariates[2, 3, 4]

	Treatment			
Location[1]	CON	EO	SEM	n
1	4.65	4.81	0.21	100
2	4.59	4.57	0.21	96
3	4.71	4.56	0.21	98
4	4.95	4.66	0.21	94
5	4.79	4.32	0.21	94
6	4.72	3.90	0.24	76
7	4.26	3.99	0.25	55

[1]Teat pairs were group from anterior to posterior along the udder line.
[2]Piglet gain = piglet weaning weight – birthweight.
[3]Lactation length effect (P < 0.0001).
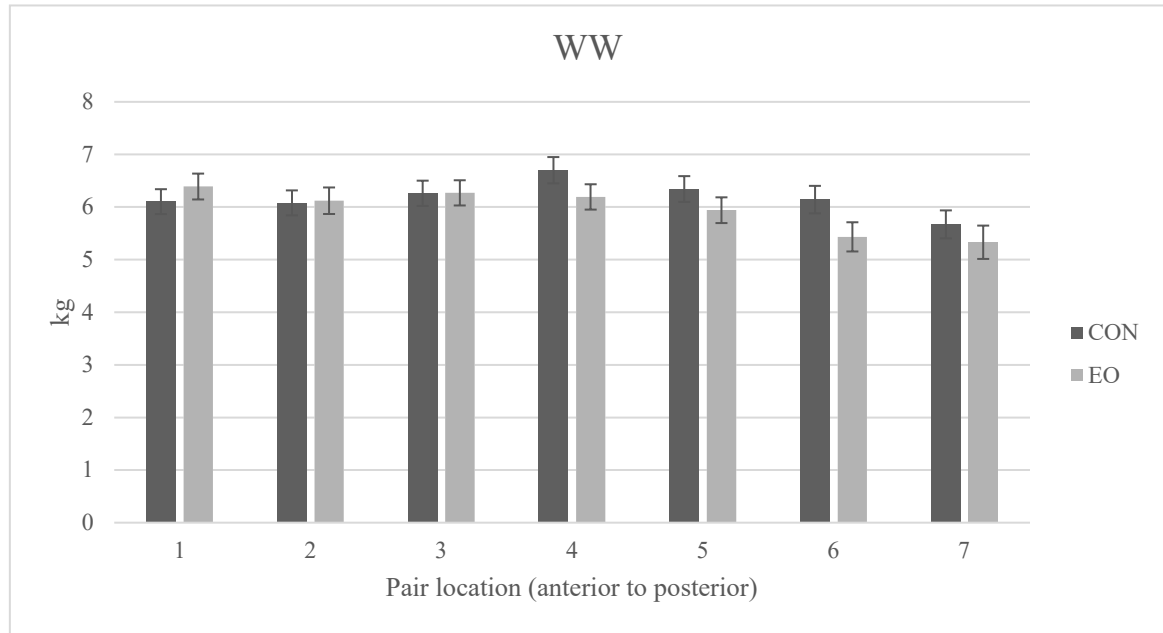[4]Linear effect on location (P = 0.01).

Figure 4.5. Effect of essential oil (EO) supplementation on piglet gain (kg) at different locations with litter size and lactation length as covariate

Table 4.13. Effect of essential oil (EO) supplementation on piglet birthweight (kg) at different locations with litter size as a covariate

| | Treatment | | | |
Location[1]	CON	EO	SEM	n
1	1.48	1.64	0.21	100
2	1.46	1.55	0.21	96
3	1.47	1.64	0.21	98
4	1.50	1.56	0.21	94
5	1.55	1.61	0.21	94
6	1.55	1.54	0.24	76
7	1.43	1.46	0.25	55

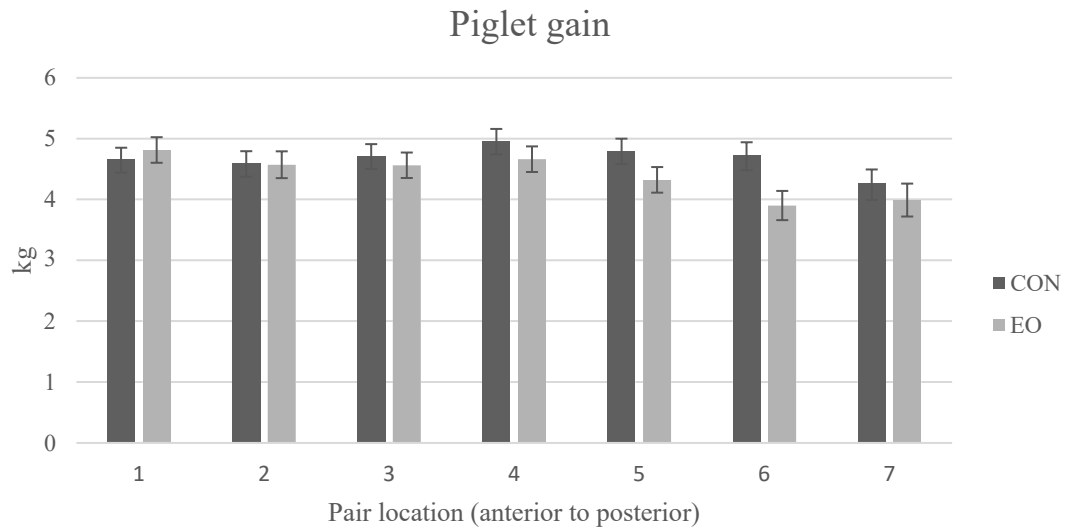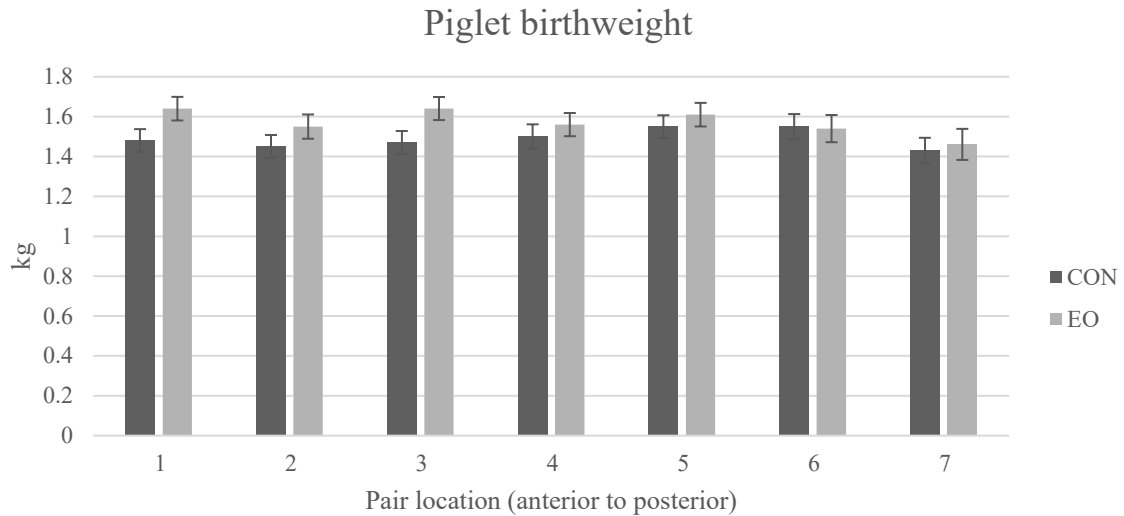[1]Teat pairs were group from anterior to posterior along the udder line.

Figure 4.6. Effect of essential oil (EO) supplementation on piglet birthweight (kg) at different locations with litter size as a covariate

4.4 Discussion

This particular essential oil supplementation (Absorbezz®) had no significant impact on any of the components found in colostrum, including immunoglobulin components. This agrees with Tan et al. (2015) when sows were supplemented with oregano essential oils at a rate of 15 mg/kg during gestation and lactation. There was no significant impact on milk components of fat, protein, and total solids. However, it did significantly impact lactose ($P = 0.04$). Milk yield was not impacted, which disagrees with Elcoso et al. (2018), who found that an essential oil supplementation of eugenol, geranyl acetate, and coriander supplemented sows had a greater milk output.

The effectiveness of an essential oil dietary addition may be heavily influenced by three things: 1) the level at which the essential oil is added, 2) the method of delivery, and 3) the essential oil used. The level of inclusion within the diets may impact the effectiveness of the essential oil in question. Balasubramaniam et al. (2016), found that protected organic acids did not affect fecal DM ($P > 0.10$); meanwhile work by Tan et al.

(2015), and Cho et al. (2014) did observe an effect on fecal DM. The second aspect that needs consideration is the mode of delivery of the product. Microencapsulation allows for substances to be delivered to specific sites of the gastrointestinal tract. This would allow for an increased efficiency in delivery within the livestock species that may increase profitability (Balasubramanian et al., 2016). Microencapsulation may increase the effectiveness of essential oils (Cho et al., 2014; Zhang et al., 2014, and Devi et al., 2016).

When supplementing a sow diet, the effects on milk output need to be considered. In this experiment, essential oil supplementation had no significant impact on the components found in colostrum, including immunoglobulin levels. This agrees with Tan et al. (2015) and Farmer (2015), who observed no significant effect on fat, protein, and total solids when sows were supplemented with oregano essential oils at a rate of 15 mg/kg during gestation and lactation. However, it did significantly impact lactose ($P = 0.04$); This is similar results to those of Matysiak et al. (2015) and Miller (2003), who determined that a blend of caracrol, cinnamaldehyde, and capsicum oleoresin increase lactose content in the milk. This could be beneficial to the piglets, in that higher lactose content may prevent hypoglycemia and potentially reduce piglet mortality (Matysiak et al., 2015). The IgA levels in colostrum were much lower than any reported value, but the concentration within milk samples is within normal values compared to other published work (Markowska-Daniel, 2010; Farmer, 2015).

In this experiment, milk yield was not impacted. This contradicts work by authors (Khajarern & Khajarern, 2002; Lipinski et al., 2012) in which there was a significant increase in daily milk production in the essential oil supplemented sows. Since milk yield influences piglet daily gain, the next logical step is to examine essential oil

supplementation on piglet gain, as well as weaning weight and birthweight. Essential oil supplementation did not significantly impact weaning weight or lactation in this study ($P > 0.50$; $P > 0.70$), but in previous research (Mellencamp et al., 2009; Matysiak et al., 2015; Lipinski et al., 2012), the piglets from litters that were supplemented had a significantly higher weaning weight and piglet gain during the lactation period. One aspect of piglet performance that was impacted was piglet birthweight. Piglet birthweight was significantly impacted by treatment ($P = 0.01$), with EO piglets having an average birthweight of 1.56 kg vs. 1.49 kg to their CON counterparts.

When comparing overall litter performance, essential oil supplementation did not affect total born, total born alive, number weaned, or mortality during lactation. While the piglets born from supplemented sows had a higher BW, it was not able to continue to significantly impact their performance to weaning.

4.5 Conclusion

There are many different aspects that may influence the potential for a sow to experience constipation. Factors such as high parity (Stanton and Carroll, 1974), gestation length (Farmer and Robert (2002), and the number of piglets the sow is carrying (Cronin et al., 1993) can all have a negative impact. Essential oils have garnered more interest in recent years, particularly for their ability to alter microbial populations. When considering dietary additions to help alleviate constipation, one must also take into consideration other impacts their inclusion may have. In this experiment, the addition of essential oils did not affect sow fecal DM % at any point in late gestation or lactation. However, it did impact piglet BW ($P = 0.01$) for sows supplemented with the essential oils. It was unable to affect the components of colostrum or milk, except for lactose ($P = 0.04$). There was no effect on

sow ADFI during lactation, which agrees with work by Tan et al. (2015) but contrasts results by Allan and Bilkei (2005), in which essential oil supplemented sows had higher ADFI. In the future, consideration for sow nutrition will continue to be a prominent concern of the industry. If dietary additions meant to alleviate a common problem can affect other aspects of lactation, analysis of current dietary ingredients or nutritional requirements may need to be examined. As the prolific sow continues to produce larger litter sizes, it is critical to the success of the industry that we continue to meet, or potentially exceed the requirements her body has during the lactation period.

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APPENDICES

Appendix I. Assay to determine the immunoglobulin content of colostrum and milk samples

The immunoglobulin ELISA kits (pig IgA [E101-102]; IgG [E101-104]; IgM [E101-117] ELISA Quantitation Kit, Bethyl Laboratories Inc., Montgomery, TX) are used to detect the immunoglobulin levels listed above in biological samples of swine, including colostrum and milk. The shelf life of the kit is six months when stored at 2-8 °C. The procedure and reagent preparation are following the manufacturer directions.

Samples used:

Colostrum and milk samples were collected from each sow during the lactation period. Colostrum was collected within 8 hr. of the onset of parturition. Sows received an intramuscular injection of 1 mL of oxytocin prior to collection. Milk samples were collected from each sow during d 14-17 of lactation. Each sow received an intravenous injection of 1 mL oxytocin (OXOJECT, Henry Schein Animal Health, Dublin, Ohio) in an ear vein. An aliquot of both colostrum and milk from each sow were centrifuged at 9,950 x g at 4°C for 20 and 10 minutes, respectively, to separate the fat from the skim layer. The fat layer was removed and discarded and the skimmed colostrum and milk samples were then centrifuged at 39,800 x g at 4°C for 45 and 20 minutes, respectively, to separate the whey fractions. The whey fractions of colostrum and milk samples were stored at -20°C until further analysis of the immunological components of IgA, IgG, and IgM.

Reagent and sample preparation:

The wash buffer and dilution buffer were prepared by combining the buffer packages with nanopure distilled water to the appropriate volume stated in the protocol. The wash buffer was prepared by diluting 50 mL of the 20X wash buffer provided by the kit into 950 mL of nanopure distilled water. The 1X dilution buffer was prepared by mixing 25 mL of the 10X wash buffer provided by the kit into 225 mL of nanopure distilled water. These reagents were mixed well prior to use. After mixing, reagents were stored at 2-8 °C, and on the day of the analysis were brought to room temperature before use.

Samples and standards were diluted to the appropriate dilution factor with the pre-made dilution buffer the day of analysis. All samples were diluted to a factor that had previously been determined in a two-day dilution factor validation. The standards were prepared in the concentrations of 0, 1.37, 4.1, 12.3, 37, 111.1, 333.3, and 1000 ng/mL for examining IgA, IgG, and IgM. The highest standard (1000 ng/mL) was created by reconstituting a provided vial of 1000 ng/mL standard with 1 mL of the dilution buffer. This represented the most concentrated standard. The other standard tubes received 300 μL of dilution buffer. The standards were serially diluted 1:3 by adding 150 μL of the 1000 ng/mL standard into the first tube containing 300 μL of dilution buffer. This tube was vortexed and inverted to allow the standard to mix well. The dilution continued by adding 150 μL of the previous standard into 300 μL of the 1X dilution buffer in the next tube until the sixth tube (1.37 ng/mL) was completed. The seventh tube contained only the 300 μL of the dilution buffer, which served as the blank.

The sample aliquots were thawed at room temperature on the day of analysis and were diluted with the sample diluent. No aliquot was thawed and used twice. All standards

and blanks were measured in duplicate. The ELISA plate map was determined and labeled prior to analysis (Figure A1-1). All of the components and the assay were conducted at room temperature.

Figure A1-1. An example ELISA plate map used for the analysis of immunoglobulins. The standards occupied columns 1 and 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 ng/mL											
B	1.37 ng/mL											
C	4.1 ng/mL											
D	12.3 ng/mL											
E	37 ng/mL		Experimental Samples									
F	111.1 ng/mL											
G	333.3 ng/mL											
H	1000 ng/mL											

Determination of dilution factor

A preliminary assay was performed prior to analysis of all collected samples. For each immunoglobulin, the manufacturer recommended a dilution factor depending on the sample type. They were as follows:

IgA: Colostrum: 1:30,000; Milk: 1:2,000

IgG: Colostrum: 1:1,000,000; Milk: 1:2,000

IgM: Colostrum: 1: 20,000

To determine the dilution factor for the samples for these experiments, a preliminary experiment was performed over two days. New standards were prepared for each day's assay. The first day, samples were diluted to 3 different dilution factors. They were as follows:
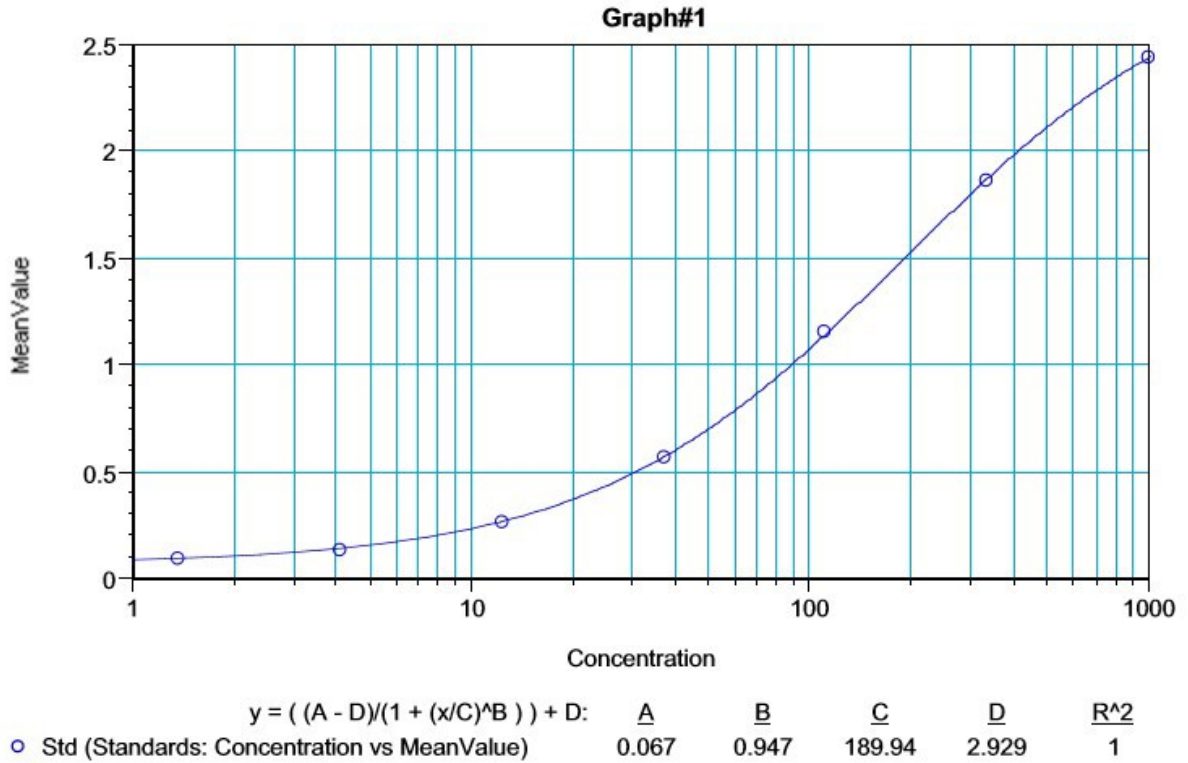
IgA: Colostrum: 30,000; 50,000; 100,000; Milk: 2,000; 5,000; 10,000

IgG: Colostrum: 500,000; 750,000; 1,000,000; Milk: 1,000; 1,500; 2,000

IgM: Colostrum: 50,000; 75,000; 100,000

The samples analyzed were from the CON sows to avoid any possible EO treatment influence. Following the absorbance readings, a dilution factor was selected for the Day 2 assay. This was determined by identifying where on the standard curve the sample absorbance fell, and then multiplying the calculated concentration by the dilution factor. Verification of the calculations was performed by cross-referencing the standard absorbance and the pre-determined concentration and matching that to the curve location. The curve provided each day is similar to that provided in Figure A1-2.

Figure A1-2. An example of the standard curve produced by the curve-fitting software used to derive unknown IgG concentrations in colostrum samples determined by the ELISA assay.

Graph#1

$y = ((A - D)/(1 + (x/C)^\wedge B)) + D$:

	A	B	C	D	R^2
o Std (Standards: Concentration vs MeanValue)	0.067	0.947	189.94	2.929	1

On Day 2, the previously determined dilution factor was utilized. For this assay, equal samples from each treatment group were analyzed. Samples were analyzed in duplicate. After the absorbance reading from the Day 2 assay, a final evaluation of the dilution factor was performed, the appropriate dilution factor identified, and that dilution factor applied to the experimental samples. The final dilution factors used for each immunoglobulin sample were as follows

IgA: Colostrum: 30,000; Milk: 10,000

IgG: Colostrum: 750,000; Milk: 1,500

IgM: Colostrum: 50,000

Assay procedures:

1) 100 μL of standard or sample were added to designated wells. The plate was covered with an adhesive plate cover strip and left at room temperature to incubate for 60 minutes.

2) After incubation, the samples and standards were aspirated from each well 4 times with the automated wash machine (Wellwash™ Microplate washer; ThermoFisher Scientific, Waltham, MA), and then dried onto a paper towel to remove residual moisture.

3) 100 μL of anti-Ig detection antibody were added to each well. The plate was covered with an adhesive plate cover strip and left at room temperature to incubate for 60 minutes.

4) After incubation, the wells were washed 4 times as described above.

5) 100 μL of HRP (streptavidin-conjugated horseradish peroxidase) solution were added to each well. The plate was covered with an adhesive plate cover strip and left at room temperature to incubate for 30 minutes.

6) After incubation, the wells were washed 4 times and dried on a paper towel.

7) 100 μL of TMB (3, 3, 5, 5'-tetramethylbenzidine) Substrate Solution were added to each well. The plate was left uncovered, in the dark, at room temperature for 30 minutes.

8) The reaction was stopped by adding 100 μL of Stop Solution to each well. The plate was tapped slightly to mix the stop solution within the wells. A lint-free tissue wiped the underside of the wells. A plate-reader (Spectramax 250, Molecular Devices Co., Sunnyvale, CA) located in the Department of Animal

and Food Science, University of Kentucky, read the plate at the wavelength of 450 nm. The plates were read within 30 minutes after the stop solution was added to the wells.

Calculation of results:

The plate required the use of curve-fitting software, and fitting the curve with a 4-parameter curve fitting equation. The software calculated the mean concentrations within each well. From the standard curve, and calculated concentration results, immunoglobulin content of each sample was determined by multiplying the calculated mean concentration by the dilution factor used, and the results are reported in mg/mL (Table A1-1). Each sample was calculated using the equation of the standard curve obtained from the same plate. An example curve output is provided in Figure A1-2.

Table A1-1. An example table used to help determine the ideal dilution factor for samples. This was part of the IgG dilution factor validation for colostrum samples. The concentration was provided by the curve-fitting software.

Sample #	TRT	Avg. Abs. nm	Avg. Concentration ng/mL	CV, %	Dilution Factor	Concentration, mg/mL
1	1	1.453	146.001	3.217	750,000	109.500
2	1	1.223	102.254	4.380	750,000	76.691
3	2	0.767	45.215	1.637	750,000	39.911
4	2	1.319	118.815	1.205	750,000	85.344

Figure A1-2. An example of the standard curve produced by the curve-fitting software used to derive unknown IgG concentrations in colostrum samples determined by the ELISA assay.

$$y = ((A - D)/(1 + (x/C)^B)) + D:$$

	A	B	C	D	R^2
○ Std (Standards: Concentration vs MeanValue)	0.067	0.947	189.94	2.929	1
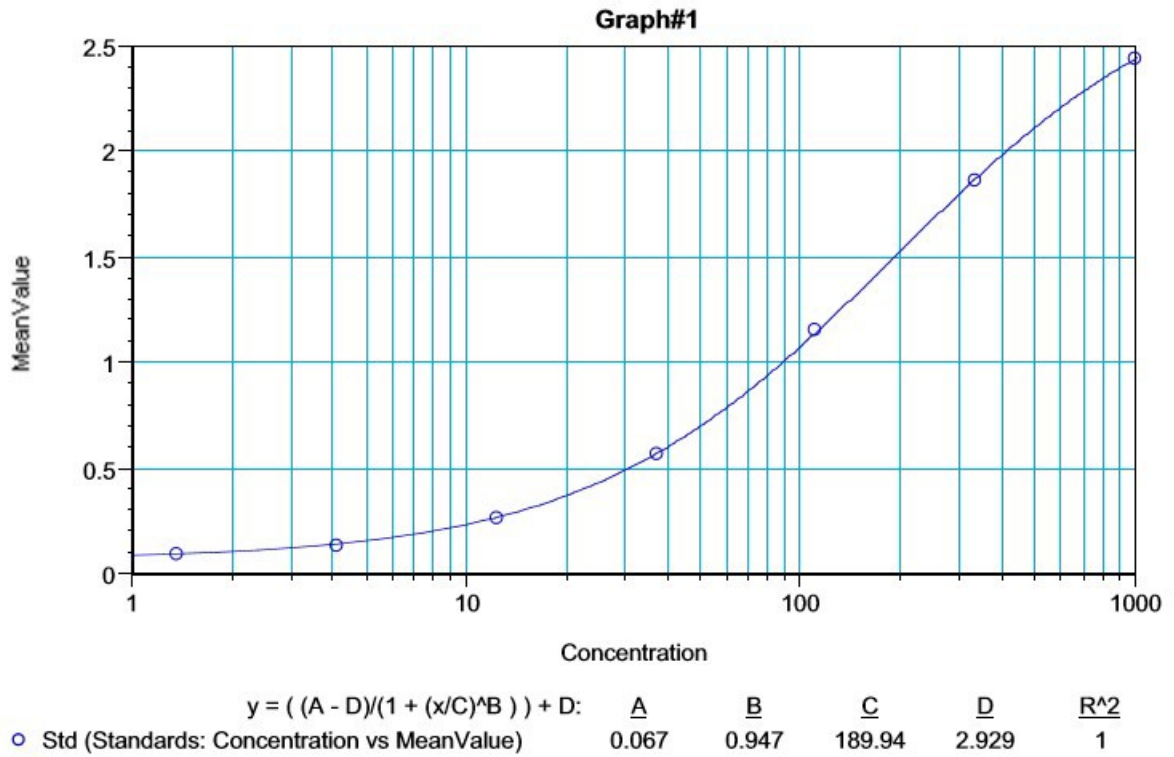
Appendix II. Assay to determine titanium dioxide levels in swine fecal and diet samples

Experiment 2 of Chapter 3 involved adding the essential oil product as a dietary ingredient and an additional response measure that was considered to be measured was the effect it had on apparent total tract digestibility (ATTD). In order to analyze ATTD, a marker was added to the diet. For this experiment, titanium dioxide was added to the diets at a rate of 0.3%. This appendix describes the process used to validate the procedure for swine fecal samples and diets that Fowler (2018) developed for equine fecal samples and diets.

Fecal Trial 1

The first trial was performed to gain an understanding of the methodology used, as well as verify that the stock solution was concentrated enough to create a standard curve that the samples being analyzed would fall within. The steps of the procedure were:

Reagents: Distilled deionized water (Nanopure); 30% hydrogen peroxide (H_2O_2); ammonium sulfate (($NH_4)_2$ SO_4); concentrated sulfuric acid (H_2SO_4); contrex acidic liquid detergent.

Equipment: Quartz crucibles; 250 mL volumetric flasks; tall beaker; small funnel; 1.5 mL cuvettes; volumetric pipettes; repeater pipette; acid-resistant repippetter; 250 mL FOSS digestion tubes (Hillerod, Denmark); FOSS Tecator Digestor (Hillerod, Denmark); Exhaust manifold; Condenser apparatus; fume hood; squirt bottle; ash oven; kimwipes;

spectrophotometer (ThermoScientific, Waltham, MD); chemical-resistant glove; tongs; parafilm; needle.

Sample preparation:

1) Dry samples overnight in a 55 °C oven to remove any excess moisture.

2) Weigh 0.15 g of dried sample into quartz crucibles in duplicate.

3) Ash the samples overnight at 600 °C in an ash oven.

4) Add 1 g of ammonium sulfate ($(NH_4)_2 SO_4$) to FOSS 250 mL digestion tubes.

5) Transfer the contents of the crucible to the 250 mL FOSS digestion tubes. Wash down the sides of the crucible and the tubes with nanopure water to ensure transfer of all of the sample.

6) Add 13 mL of concentrated sulfuric acid (H_2SO_4) to the digestion tubes.

7) Place tubes in the FOSS Digestor 2520 and place the exhaust manifold on top of the tubes.

8) Set the machine at 420 °C for 3 hours. The machine will take approximately 1 hour to come up to temperature.

9) Label volumetric flasks (250 mL flasks were used here while Fowler [2018] used 50 mL flasks) with corresponding labels to the digestion tubes. Add 10 mL of 30% hydrogen peroxide (H_2O_2) to each flask. If preparing flasks prior to 30 minutes before the digestion is complete, place flasks in refrigerator to keep cool, which keeps the peroxide fresh. Fresh peroxide is required for complete reaction to occur.

10) After 3 hours of boiling, remove tubes from digestor and allow to sit in fume hood until they stop fuming.

11) Pour the contents into the 250 mL flasks that contain the hydrogen peroxide. First squirt a small amount of nanopure water into the tube to dilute the acid. Pour off the tube into the flask and rinse with nanopure water.

12) Let flasks cool down, dilute to volume and mix. Parafilm the flasks and pop a hole in the film with a needle. Mix by inverting and shaking at least 3 times. Allow pressure built up in flasks to be released through the needle hole after each inversion to avoid explosions.

13) Let flasks sit overnight to allow particles to settle to the bottom.

14) Transfer an aliquot of each sample, standards, and blank into cuvettes. Measure aliquots on a spectrophotometer at 410 nm with the blank standard (0 mg/mL Ti) as the blank used to zero the spectrometer. Measure absorbance at least 3 times in a row before recording absorbance.

Standard curve preparation:

1) Pipette 0, 0.5, 1, 1.5, and 2 ml of the standard titanium solution (0.5 mg/ml) into individual 50 ml volumetric flasks.

2) Add concentrated sulphuric acid to each flask so that the combined volume is 10 ml.

Standard sol'n (mL)	H_2SO_4 added (mL)	TiO_2 Concentration (mg/mL)
0	10	0
0.5	9.5	0.005
1.0	9	0.010
1.5	8.5	0.015
2	8	0.020

3) Add 10 ml of 30% H_2O_2 to each flask and dilute to volume with nanopure water.

4) Measure aliquots on a spectrophotometer at 410 nm to obtain a calibration curve.

Trial 1 results

Trial 1 followed the protocol outlined above. The standard curve is found in Figure A2-1. The results from fecal samples are found in Table A2-1 and includes the CV % between duplicates. Calculation of ATTD % is as follows:

$$\text{ATTD (\%)} = 1 - \frac{\text{Nutrient}_{feces}}{\text{Nutrient}_{feed}} \times \frac{\text{Marker}_{feed}}{\text{Marker}_{feces}} \times 100 \left[\right]$$

Theoretical expectations were as follows: expected titanium determination (diet): 0.3%; expected titanium determination (fecal): 1.50-3%. The expected value is based an anticipated digestibility of 80-90%.

An example calculation (80% digestibility):

1-0.80 = 0.20

$$\frac{0.30 \text{ (feed marker \%)}}{0.80 \text{ (digestibility hypothesis)}} \times 100 = 1.50 \text{ \% TiO}_2 \text{ in fecal samples}$$

The calculation for TiO_2 determination that will be applied to all trials in this appendix are as follows:

Determination of TiO_2 (mg/mL): this was calculated using the absorbance read and the

standard curve produced.

$$TiO_2 \text{ mg/mL} = \frac{(Absorbance-y \text{ intercept})}{slope}$$

$$TiO_2\% = \frac{(TiO_2 \text{ mg/mL}) * final \text{ volume}}{Sample \text{ wt. (g)}}$$

Figure A2-1. Standard curve for titanium dioxide determination in swine fecal samples
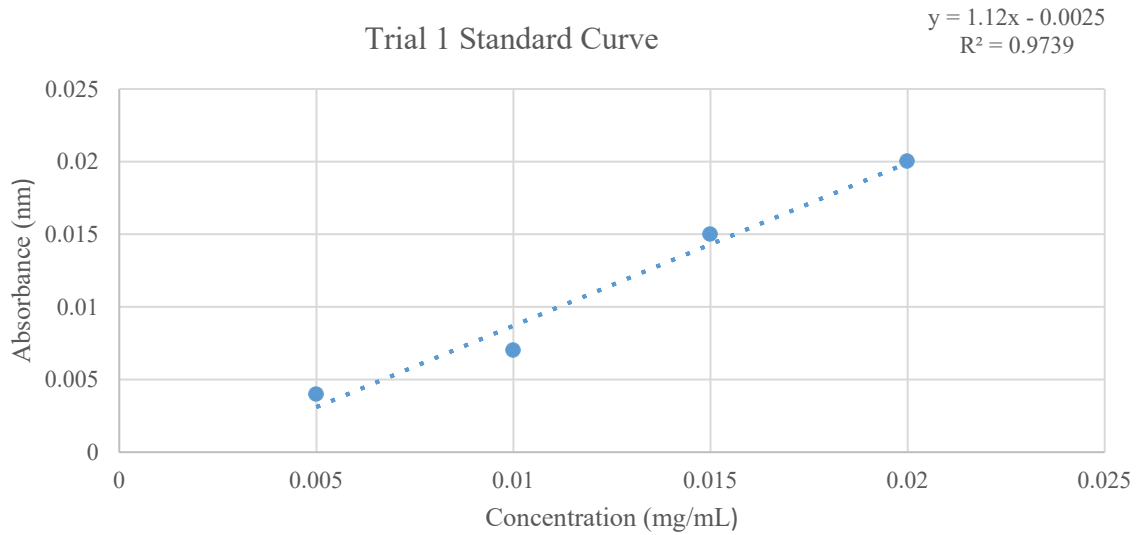

Trial 1 Standard Curve

y = 1.12x - 0.0025
R^2 = 0.9739

Table A2-1. Titanium dioxide determination in swine fecal samples[1]

Sample[2]	Avg. Abs. nm	Avg. TiO$_2$, mg/mL	Avg. TiO$_2$, %[3]	CV, %
1	0.184	0.188	20.226	17.875
2	0.004	0.005	0.653	28.195
3	0.124	0.127	20.437	6.509
4	0.169	0.172	26.498	1.335

[1]Samples were randomized when placed in the digestor to keep duplicates separate
[2]Samples were analyzed in duplicate
[3]Theoretical expectation in fecal samples: 1.50-3%

From the results in Trial 1 found in Figure A2-1 and Table A2-1, two corrections needed to occur. First, the stock concentration of the stock solution needed to increase. The standard curve created by the samples used with a stock solution of 0.005 was too low for fecal samples, as the absorbances are well above the fifth standard. Secondly, a set of samples needed to be spiked to test for titanium recovery in the fecal samples as a method of validation for this assay.

Fecal Trial 2

This assay utilized an updated stock solution, containing 1.25 mg/mL TiO_2. An additional goal was to reduce the CV % between duplicate samples, before spiking individual samples. The standard curve for this trial is found in Figure A2-2, and results are found in Table A2-2.

New standard: 1.25 mg/mL TiO_2

Standard sol'n (ml)	H_2SO_4 added (ml)	TiO_2 Concentration (mg/ml)
2	8	0.010
4	6	0.020
6	4	0.030
8	2	0.040

1) Add 10 ml of 30% H_2O_2 to each flask and dilute to volume with nanopure water.
2) Measure aliquots on a spectrophotometer at 410 nm to obtain a calibration curve.

Figure A2-2. Standard curve for titanium dioxide determination in swine fecal samples
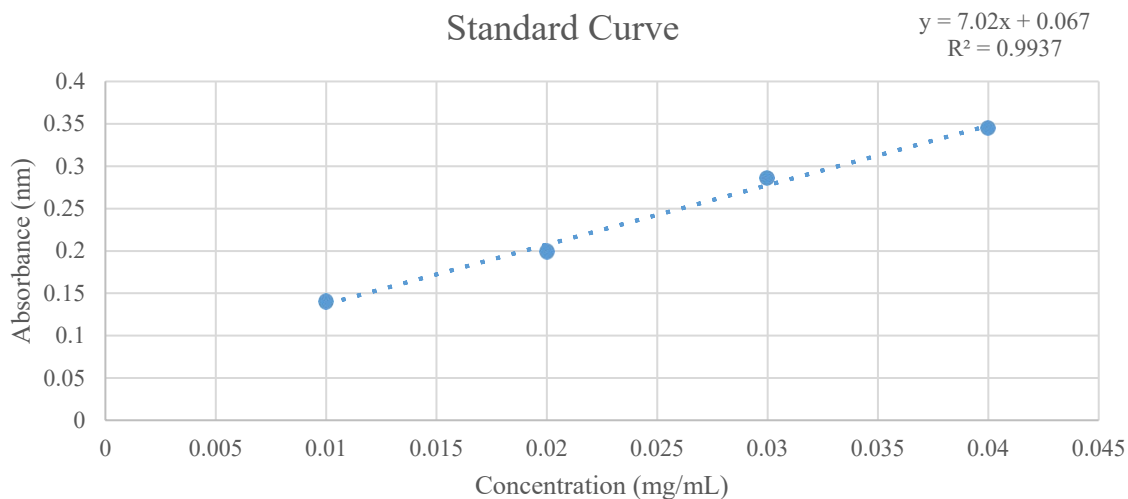

Standard Curve

$y = 7.02x + 0.067$
$R^2 = 0.9937$

Table A2-2. Titanium dioxide determination in swine fecal samples[1]

Sample[2]	Avg. Abs. nm	Avg. TiO$_2$, mg/mL	Avg. TiO$_2$, %[3]	CV, %
1	0.215	0.021	3.238	2.876
2	0.169	0.014	2.415	5.914
3	0.187	0.017	2.750	13.616
4	0.171	0.015	2.476	2.011
5	0.223	0.022	3.533	12.183
6	0.148	0.011	1.693	14.265
7	0.152	0.012	1.822	12.099
8	0.160	0.013	2.158	1.089

[1]Samples were randomized when placed in the digestor to keep duplicates separate
[2]Samples were analyzed in duplicate
[3]Theoretical expectation in fecal samples: 1.50-3%

This assay was successful in reducing the CV% between duplicate samples. From the results of this assay, it appears that if anticipating approximately 1.50 – 3% levels of TiO$_2$ in the fecal samples, the results of this trial are within that estimated range. The next step

was to analyze spiked titanium samples using a known amount of added titanium dioxide in fecal samples from animals that did not consume a marker diet.

Fecal Trial 3

This trial focused specifically on spiking fecal samples by adding a known amount of titanium dioxide to the fecal sample. Calculating a high percent recovery would assist in the validation of this assay across species. A small sub-set of the fecal samples without titanium dioxide added to it were also analyzed to verify that the fecal samples that did not contain titanium dioxide.

This trial required calculating percent recovery of the spiked fecal samples. Percent recovery was calculated by dividing the concentration of (recovered)TiO_2 / (sample wt. TiO_2 added to the diets) and multiplying by 100 to create a percent TiO_2%, recovery, shown below.

$$\% \text{ recovery} = \frac{TiO_2, \text{ recovered (g)}}{\text{sample wt. } TiO_2} *100$$

From the results of this table, there is no detectable contamination of these tested fecal samples (Table A2-3). In Table A2-4, the samples that were spiked with the titanium have higher percent recoveries. The first sample, which had approximately 32% of added TiO_2 had a much higher absorbance value than the other two spike amount tested. Based off of the standard curve in Figure A2-3, that absorbance is beyond the standard curve, which makes it difficult to determine if this is a conclusive result. Since the fecal sample analysis (Table A2-3) was not producing results that produced a conclusive idea on whether this

methodology was successful in swine, the next step was to test the diet samples that were retrieved during the diet mixing process. While the goal was to initially validate this procedure by spiking samples, the next logical trial involved starting with a known percentage of titanium dioxide, in this case, what was mixed in the diet. The next 3 trials provide an overview of the methodology used as part of the validation process.

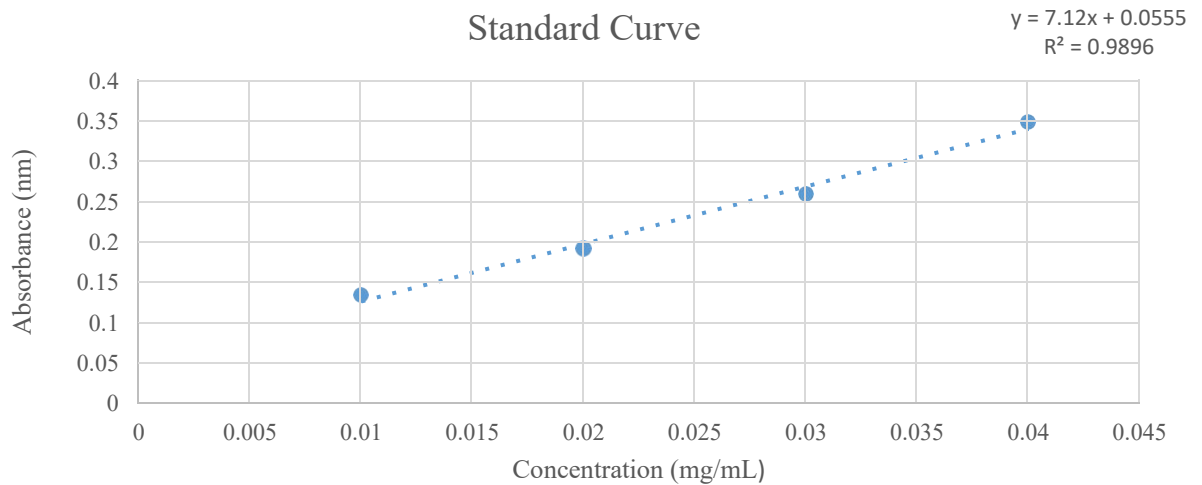Figure A2-3. Standard curve for titanium dioxide determination in swine fecal samples

Table A2-3. Titanium dioxide determination in swine fecal samples when titanium dioxide was not fed[1]

Sample[2]	Avg. Abs. nm	Avg. TiO$_2$, mg/mL	Avg. TiO$_2$, %[3]	CV, %
1	-0.003	-0.008	-1.250	-0.859
2	0.002	-0.007	-1.680	-0.552
3	0.008	-0.006	-0.949	-0.943

[1]Samples were randomized when placed in the digestor to keep duplicates separate
[2]Samples were analyzed in duplicate
[3]Theoretical expectation in fecal samples: 0%

Table A2-4. Titanium dioxide determination in spiked swine fecal samples[1]

Sample[2]	Sample wt. g	TiO$_2$ added, g	Expected recovery, TiO$_2$	Avg. Abs. nm	Avg. TiO$_2$, mg/mL	Avg. TiO$_2$, %[3]	TiO$_2$, recovered	CV, %
1	0.158	0.048	19.03	1.386	0.187	21.626	101.844	9.556
2	0.164	0.010	5.00	0.304	0.035	5.001	88.405	19.722
3	0.161	0.011	5.42	0.265	0.029	4.172	68.002	43.456

[1]Samples were randomized when placed in the digestor to keep duplicates separate
[2]Samples were analyzed in duplicate
[3]Theoretical expectation in spiked fecal samples: 5.00%, 5.42 %, 19.03% (assuming 100% recovery)

Diets Trial 1

The protocol provided by Fowler et al. (2018) recommended using a sample size of 0.15 g for both diet and fecal samples. Since there was a different size of volumetric flask used (250 mL vs. 50 mL used by Fowler), this trial analyzed the same sample size, some containing samples with added titanium dioxide, some without, and then different volume of flasks utilized. This would verify that the volume the final sample was diluted to was not affecting the overall results. The fecal samples were spiked with the following size of titanium dioxide: 0.003, 0.006, 0.012, and 0.024 g added.

Figure A2-4. Standard curve from of titanium dioxide recovery in swine diet samples
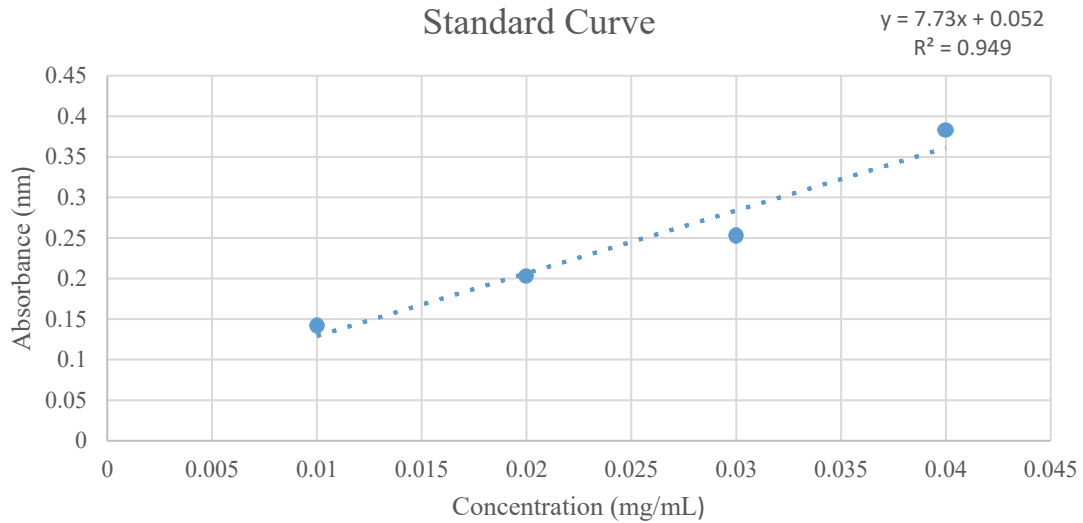
From this diet analysis trial, there were several conclusions. The first, the volume did not seem to affect the overall concentration reported (Table A2-6, A2-7). Therefore, there is no longer a concern that the volumetric flasks volume was causing the final product to become too dilute. Additionally, spiking the diets did not seem to be effective in determining a percent recovery (Table A2-5). Based off the diet formulations for this experiment, it can be hypothesized that there may be approximately 1.50-3% of TiO_2 within the diet samples, this is assuming that there is approximately 80-90% digestibility of the diet in the animal. Since the values are still negative, or similar to that, the next step in validating the methodology was to examine a combination of different sample sizes, different amounts of acids, and different levels of ammonium sulfate additions.

Following discussion with another lab, it was recommended that the sample size for the diets increase to 3-5 g of sample, as it was hypothesized that the sample sized used was not large enough for correct titanium detection.

Table A2-5. Titanium dioxide recovery in spiked diet samples with different spike amounts[1]

Sample[2]	Sample wt. g	TiO$_2$ added, g	TiO$_2$ in sample[3]	% TiO$_2$ in diet[4]	Avg. Abs. nm	Avg. TiO$_2$, mg/mL	Avg. TiO$_2$, %[3]	TiO$_2$, measured	CV, %
1	0.154	0.003	0.046	4.60	0.044	-0.000	-0.161	-0.011	-2.450
2	0.149	0.006	0.239	2.39	0.199	0.019	3.199	0.510	10.195
3	0.156	0.013	0.048	4.80	0.093	0.005	0.857	0.076	7.500
4	0.155	0.024	0.067	6.70	0.274	0.029	4.652	0.221	4.909

[1]Samples were randomized when placed in the digestor to keep triplicates separate
[2]Samples were analyzed in triplicate
[3]This calculation accounts for the percent of titanium added to the sample + the percent of titanium dioxide in the diet.
[4]Assuming an 80-90% digestibility.

Table A2-6. Titanium dioxide determination in diets diluted to different final volumes[1,4]

Sample[2]	Final volume, mL	Avg. Abs. nm	Avg. TiO$_2$, mg/mL	Avg. TiO$_2$, %[3]	CV, %
1	100	0.016	-0.004	-0.297	16.781
2	200	0.004	-0.006	-0.821	7.438

[1]Samples were randomized when placed in the digestor to keep triplicates separate
[2]Samples were analyzed in triplicate
[3]Theoretical expectation in diets: 4.5% assuming a 0.15 g sample
[4]Volumes of volumetric flasks used: 100 mL, 200 mL

Diets Trial 2

As a final attempt to validate this methodology, this trial analyzed different sample sizes of diets (0.15 g; 0.50 g; 1.50 g; 4.50 g). The reagent amounts would not change. This would help determine if the sample size is simply not large enough to detect any level of titanium dioxide. These samples were analyzed in triplicate, and samples from each diet were utilized. In addition, a fifth standard was added as part of the standard curve, to capture samples that may be low in absorbance and concentration.

In analyzing the results, this trial provided the most positive numbers across all diet trials. Interestingly, the 4.50 g samples were the closest to the 0.3% that the diet contains (Table A2-8). In the future, should more validation attempts occur in swine, a larger sample size will produce better results. Additionally, subsets of both diets and fecal samples should be sent off for analysis in a validated lab that performs titanium dioxide analysis. This will provide the investigator with expected values and will provide a better idea on sample size and methodology in the future.

Table A2-7. Titanium dioxide determination in diets diluted to 250 mL[1]

Sample[2]	Avg. Abs. nm	Avg. TiO$_2$, mg/mL	Avg. TiO$_2$, %[3]	CV, %
1	0.012	-0.005	-0.844	6.048
2	0.014	-0.004	-0.822	0.000
3	0.009	-0.006	-0.894	6.560
4	0.008	-0.006	-0.931	8.501

[1]Samples were randomized when placed in the digestor to keep triplicates separate
[2]Samples were analyzed in triplicate
[3]Theoretical expectation in diets: 4.5% assuming a 0.15 g sample

Updated standard curve

Standard sol'n (ml)	H$_2$SO$_4$ added (ml)	TiO$_2$ Concentration (mg/ml)
1	9	0.005
2	8	0.010
4	6	0.020
6	4	0.030
8	2	0.040

1) Add 10 ml of 30% H$_2$O$_2$ to each flask and dilute to volume with nanopure water.
2) Measure aliquots on a spectrophotometer at 410 nm to obtain a calibration curve.

Figure A2-6: Standard curve for titanium dioxide determination in diets[1]

Standard Curve

$y = 8.8316x + 0.0313$
$R^2 = 0.9717$
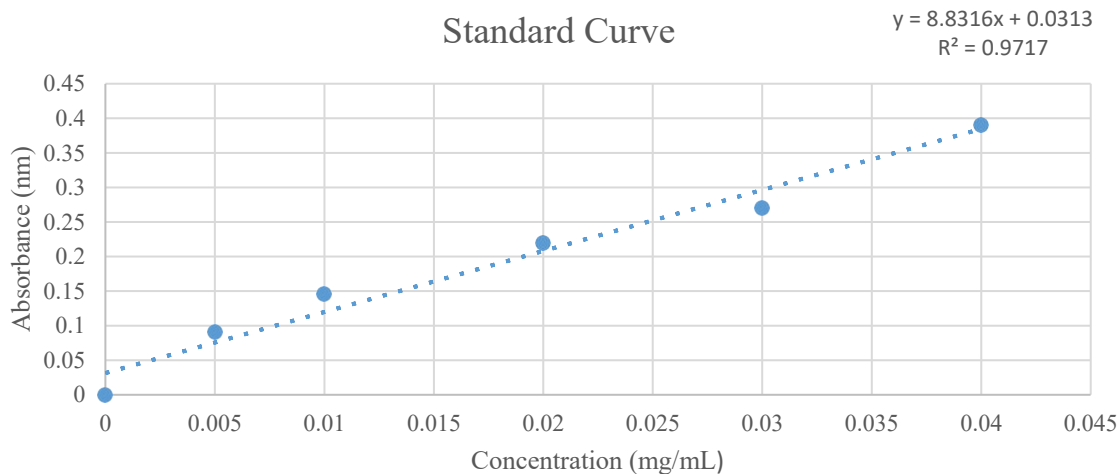
Table A2-8. Titanium dioxide determination in diets utilizing different sample sizes

Sample[2]	Sample size, g	Avg. Abs. nm	Avg. TiO$_2$, mg/mL	Avg. TiO$_2$[4], %	CV, %
1[3]	0.15	0.023	-0.000	-0.148	0.000
2	0.50	0.064	0.004	0.186	27.294
3	1.50	0.164	0.015	0.249	5.381
4	4.50	0.305	0.031	0.172	9.716
5	0.50	0.073	0.005	0.236	12.732
6	1.50	0.186	0.018	0.292	6.435
7	4.50	0.271	0.028	0.150	19.968

[1]Samples were randomized when placed in the digestor to keep triplicates separate
[2]Samples were analyzed in triplicate
[3]Sample 1 did not have any duplicate samples due to space
[43]Theoretical expectation in diet samples: 0.3%

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Dierking, Shannon, Jim Monegue, and Merlin D. Lindemann. 2018. Supplementation of essential oils in lactating sow diets and its effect on weaning weight and milk composition. Animal and Food Sciences Graduate Student Association Poster Symposium, 2018, Lexington, KY.

Awards

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