STIMULATING ESTRUS AND OVULATION IN LACTATING SOWS AND CONSEQUENCES FOR PIG GROWTH

by

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B.S., Kansas State University, 2009
M.S., Kansas State University, 2012

AN ABSTRACT OF A DISSERTATION

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Abstract

A total of 188 sows and their litters were used in 2 experiments to evaluate methods to induce estrus and ovulation in lactating sows and effects on pig growth. In Exp. 1, an altered suckling method (ALT) was designed to combine split-weaning and intermittent suckling as a means to reduce the suckling stimulus in primi- and multiparous sows during the last week of lactation (d 18 to 25). The ALT sows were also removed for daily boar exposure. The ALT treatment produced lactational estrus in 75% and 95% of primiparous and multiparous sows, respectively. The ALT sows were in estrus earlier ($P < 0.01$) than controls post-farrowing, with no effect on subsequent reproductive performance. From d 18 to 32, the ALT treatment benefited ($P < 0.01$) growth of lightweight pigs but decreased ($P < 0.01$) BW gain of heavyweight pigs, resulting in overall similar growth. However, variation in BW was reduced ($P < 0.01$) by 50% for ALT litters. In Exp. 2, varying suckling reduction strategies were applied to boar-exposed lactating sows. Overall, 76% of sows in suckling reduction treatments expressed estrus in lactation. Split-weaned and ALT sows performed reproductively similar to controls, whereas sows with daily litter separation or a single 24 h litter removal tended ($P < 0.10$) to have reduced conception rates versus controls or split-weaned sows. Reduced suckling treatments differed in their ability to induce lactational estrus and impact on pig BW gain immediately post-weaning. However, no evidence was found of benefit for pig growth to market weight or litter BW variation. Four additional experiments using 902 nursery pigs were conducted to test the efficacy of potential detoxifying agents against deoxynivalenol (DON) in swine diets. The effects of DON were not offset by adding an algae-modified montmorillonite clay nor by a proprietary blend of preservatives and clays. However, hydrothermally treating DON-contaminated diets

with sodium metabisulfite modified the structure of DON to a non-toxic DON-sulfonate adduct and restored nursery pig growth via improved ($P < 0.05$) ADG, ADFI and G:F.

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Chapter 1 - Ovulation Induction in Lactating Sows: Should We Reconsider?

INTRODUCTION

On swine farms, optimal reproductive performance centers upon ideal management during the farrowing and lactation period through the onset of estrus and insemination. While substandard management of sows post-insemination and during pregnancy can impair reproductive performance (Knox, et al. 2014), discussion of these factors goes beyond the scope of the present review. Swine producers and genetic companies have consistently emphasized increasing the number of pigs/sow/yr, which over time has resulted in marked improvements in sow farm productivity. During the 1990's in the United States, increasing the number of litters/sow/yr was accomplished by reducing the farrowing interval using a segregated early weaning system (Dial, et al. 1995; King et al., 1998). This practice increased productivity by reducing lactation lengths while concurrently preventing vertical transmission of disease between sows and piglets (Dritz et al., 1994). However, Soede et al. (2009) and Varley (1982) concluded that short lactations (<21 d) negatively impact post-weaning follicular development, wean-to-estrus interval (WEI), and ovulation response and contribute to reduced subsequent farrowing rate and litter size. Also, due to concerns that early-weaned pigs acquire negative behavioral patterns that persist into the finishing period (von Borell, 2000), European Union legislation has applied a minimum weaning age of 21 d.

The traditional mindset in the swine industry is that weaning is the start of the reproductive cycle in sows. However, more recently breeding sows during lactation has been proposed as an alternative approach which may also increase annual sow productivity (Kemp and Soede, 2012a). If sows conceive while lactating, farrowing interval and herd non-productive

days may be decreased, thereby increasing the number of litters/sow/yr (Kirkwood and Thacker, 1998). Attempts to breed sows during lactation 20 to 40 yr ago yielded inconsistent results (Crighton, 1970a; Stevenson and Davis, 1984a; Newton et al., 1987a; Costa and Varley, 1995), and the longer WEI in sow genotypes at the time may have contributed to the limited success (Aumaitre et al., 1976; Britt and Levis, 1983). Partly due to genetic selection and improved management, contemporary sow lines are less likely to have extended WEI and appear to be more receptive to lactational ovulation induction (Kemp and Soede, 2012a).

This review will first discuss the underlying reasons why sows generally remain anestrus during lactation, followed by an explanation of why some sows overcome inhibitory factors and spontaneously ovulate during lactation. A deeper examination of the physiological and endocrine processes related to lactational anestrus will be presented, as well as an overview of different techniques which have been attempted to stimulate estrus in lactating sows. Overall, this review aims to reconsider the long-held view that weaning should occur before re-breeding.

LACTATIONAL ANESTRUS

According to Warnick et al. (1950) and Baker et al. (1953), sows commonly display an anovulatory estrus in the first few days following parturition. This initial estrus is generally undetected and probably results from placental estrogens. However, after the initial 72 h post-partum, lactation is established and teat stimulation by the piglets and the proximity of the piglets suppresses pulsatile luteinizing hormone (LH) secretion by inhibiting the gonadotropin-releasing hormone (GnRH) pulse generator (Kemp et al., 2009; Quesnel 2009). This inhibitory effect is mainly due to the release of endogenous opioid peptides (EOP) in the brain (Armstrong et al., 1988; De Rensis et al., 1999) and begins the series of events which ultimately prevent follicular growth and ovulation. The severity of LH inhibition may also be impacted by the energy balance

of the sow as decreased peripheral LH concentrations were reported in primiparous sows subjected to feed restriction versus those on a high feeding level (Tokach et al., 1992; Quesnel and Prunier, 1998). As lactation progresses, LH pulsatility is gradually restored, which may be attributed to decreased suckling frequency combined with an increase in pituitary responsiveness to GnRH and increases in releasable LH pools within the pituitary (Sesti and Britt, 1993; Quesnel 2009; Soede and Kemp, 2015). In conjunction with the restoration of LH release, follicle diameter increases as lactation progresses (reviewed by Britt et al., 1985), but follicles usually do not reach pre-ovulatory size until after weaning (Lucy et al., 2001). While not likely to occur during the first 14 d of lactation, occasionally some sows escape the suckling-induced LH suppression, develop pre-ovulatory size follicles (~8 mm), and ovulate during lactation (Langendijk et al., 2009).

SPONTANEOUS OVULATION DURING LACTATION

While sows generally remain anestrus until weaning because of the suckling-induced LH suppression, some sows overcome the lactational inhibition and ovulate before weaning (Kemp et al., 2009). Spontaneous lactational ovulation appears to be more common in contemporary hyperprolific sow lines. Selection pressure has emphasized increased prolificacy and shorter rebreeding intervals (Rutherford et al., 2013; Quesnel et al., 2015), and contemporary sow lines commonly farrow 15 or more live born and are less likely to display prolonged WEI, even in the face of nutrient restriction (reviewed by Kemp and Soede et al., 2012a). According to Kemp and Soede (2012a), spontaneous lactational ovulation is most likely to occur in multiparous sows and if a low number of piglets are nursing. High lactation feed intake and group lactation systems are also predisposing factors. The incidence of spontaneous ovulation also appears to increase with weaning age. In fact, Downing et al. (2012) observed that when sows were weaned on d 25

and 29 post-farrowing, 12 and 17% spontaneously ovulated during lactation, respectively. Terry et al. (2014) observed spontaneous ovulation in 5% of sows by d 18 and in 24% of sows between d 18 and 29 of lactation in multiparous sows. Taken together, these results indicate that the contemporary sow is more predisposed to ovulate during lactation. Sows ovulating during lactation are likely mistaken for delayed estrus sows, contribute to increased variation in WEI, and as a result may be culled. Spontaneous lactational ovulations have the potential to diminish farm reproductive performance. On the other hand, the occurrence of lactational ovulation also signifies the contemporary sows' receptivity to manipulation by additional stimuli. This receptivity will receive additional attention in later sections of the review.

PHYSIOLOGICAL AND ENDOCRINE PROCESSES INVOLVED

Uterine Involution

The time needed for uterine tissue repair and regeneration (involution) following parturition may also impact rebreeding performance (as reviewed by Polge, 1972). Uteri rapidly decline in length and weight during the first week post-farrowing and continue to regress more slowly until 21 to 28 d postpartum (Palmer et al., 1965). Based on histological observations, the endometrium appears to degenerate during the first 7 d postpartum, but regenerative changes begin around 7 d and epithelial tissue repair appears to be complete by 21 d (Palmer et al., 1965). Since ovulation and fertilization rate are not impaired by short lactation lengths (Varley, 1982; Marstetler et al., 1997), the reduced embryo survival and decreased litter size often observed in early-weaned sows (< 21 d) is likely attributed to high embryo mortality between 9 and 20 d after conception. Losses during this implantation period probably occur as a result of the reduced ability for the embryo to make a successful placental attachment to the not fully repaired endometrium (Varley and Cole, 1978). The suckling stimulus also appears to be involved in

uterine involution as Graves et al. (1967) reported that involution occurred more rapidly in suckled sows compared to sows who were weaned after short lactations. Yet, it is unknown whether the negative consequences of short lactation lengths associated with compromised uterine recovery are as severe in sow genotypes used today, as genetic selection has led to marked changes in reproductive performance and body composition.

Endocrine Consequences of Suckling

Endogenous Opioid Peptides (EOP): During lactation, stimulation of the teats by the piglets and their proximity to the sow elicit neuroendocrine reflexes that induce the release of endogenous opioids in the central nervous system of the sow (Quesnel, 2009). These neuropeptides have a morphine-like biological activity and include compounds such as endorphins, enkephalins, and dynorphins, which are the natural ligands for receptors that also bind opiates (Estienne and Barb, 2005). In general, EOP suppress gonadotropin secretion. In a study in lactating sows (De Rensis et al., 1999), administration of morphine, an EOP agonist, decreased LH and prolactin secretion during lactation. In contrast, the administration of naloxone, an opioid antagonist, increased basal LH secretion and pulsatility (Barb et al., 1986; Mattioli et al., 1986) in lactating sows. The ability of naloxone to counteract suppression of LH during lactation is consistent with a role for EOP in obstructing reproductive activity in lactating sows (De Rensis et al., 1993).

Gonadotropin-Releasing Hormone (GnRH): Inhibitory inputs from EOP lead to suppression of GnRH pulsatility and decrease pituitary sensitivity to GnRH (Quesnel and Prunier, 1995). Suckling-induced GnRH inhibition thereby restricts accumulation of peripheral LH and suppresses LH pulsatility (Soede and Kemp, 2015), whereas follicle stimulating hormone (FSH) secretion during lactation seems to be impacted more by inhibin (produced by

follicles > 3 mm; Noguchi et al., 2010) and less by suckling effects on GnRH. Sesti and Britt (1993) reported a gradual increase in basal gonadotropin secretion beginning after the first week of lactation, which led to progressively increasing follicular development over the course of lactation. Moreover, Sesti and Britt (1993) detected a lessened sensitivity to the inhibitory effects of suckling in multiparous versus primiparous sows. Further proof that suckling-induced GnRH suppression provides the initial block upstream is provided by Stevenson et al. (1981) and reviewed by Britt et al. (1985), demonstrating that both the ovary and pituitary glands remain responsive as exogenous GnRH injections stimulate the release of LH and result in ovulation. This receptivity has led to multiple attempts to predictably induce ovulation using administration of pregnant mare serum gonadotropin (PMSG) or human chorionic gonadotropin (hCG) during lactation (Guthrie et al., 1978; Hausler et al., 1980; Hodson et al., 1981; Kirkwood and Thacker, 1998). Applied at various stages of lactation (d 7 to 28 post-partum), these attempts at hormonal induction of estrus have resulted in high rates of lactational ovulation. Nevertheless, subsequent pregnancy rates have been poor, which may be attributed to an unfavorable uterine environment for placentation to occur (Hodson et al., 1981).

Luteinizing Hormone (LH): After farrowing, circulating concentrations of progesterone and estrogens fall and LH secretion increases immediately (Quesnel 2009). However, by 72 h post-parturition, lactation is fully established and LH secretion is once again suppressed (De Rensis et al., 1993). This leads to a quiescent period where the ovaries remain inactive for about 10 d, resulting in only small follicles (1 to 2 mm) in the antral follicle pool (Britt et al., 1985). Low peripheral LH concentrations during early to mid-lactation are related not only to suckling-induced GnRH inhibition, but also to the limited pituitary LH pools which are depleted just after farrowing. Jones and Stahly (1999) demonstrated that as lactation progresses, pituitary LH stores

are restored and the sow develops a greater capacity to mount an LH surge in response to estrogens (Bevers et al., 1981; Sesti and Britt, 1993). Accordingly, the absolute amount of LH released during the pre-ovulatory surge is significantly lower after 21 d of lactation as compared to sows weaned after a 35 d lactation (Edwards and Foxcroft, 1983). In fact, sows weaned immediately post-farrowing (Varley and Foxcroft, 1990) or weaned after short lactations (Ryan and Raeside, 1991; Castagna et al., 2004) are more likely to develop cystic follicles, which is likely related to the absence of an LH surge caused in part by insufficient pituitary LH pools (Gerritsen et al., 2014). The occurrence of ovarian cysts reduces overall herd performance by causing reduced conception rates, irregular estrous cycles, and behavioral changes (Castagna et. al, 2004).

Prolactin: Prolactin is an essential hormone for lactogenesis (Farmer et al., 1998) and plays an important role in various other reproductive processes in mammals (Dusza and Tilton, 1990). Elevated prolactin levels in the pre-parturient period are critical for the onset of lactation (Taverne et al., 1982). As reviewed by Alonso-Spilsbury et al. (2004), basal prolactin levels are lower during lactation than around farrowing, but each suckling event elicits a temporary increase in prolactin concentration which gradually returns to basal levels. Prolactin peripheral concentrations decrease over the course of lactation, likely attributed to the decrease in suckling frequency, yet remain higher than during the estrous cycle (Stevenson et al., 1981; Edwards and Foxcroft, 1983). Rapid declines in prolactin occur at weaning (Foxcroft et al., 1987), in zero-weaned sows (De Rensis et al., 1993) and in response to partial weaning or temporary separation from the litter (Bevers et al., 1981; Stevenson et al., 1981).

Discussion remains around whether prolactin plays a role in lactational anestrus because an inverse relationship between prolactin and LH has generally been reported (Quesnel and

Prunier, 1995). One possible hypothesis to explain this inverse relationship is that suckling stimulates the secretion of prolactin which in turn suppresses LH secretion. Booman et al. (1982) treated sows with prolactin for a 24 h period after weaning and found reductions in mean plasma LH, basal LH, and frequency of LH pulses. In lactating rats during early lactation, Smith et al. (1978) attributed suckling-mediated EOP to a greater suppression of gonadotropins; though during later lactation, prolactin played a larger role in gonadotropin suppression.

Alternatively, Mattioli et al. (1986) proposed that the inverse prolactin/LH relationship can be attributed to the suckling-induced release of EOP, which suppresses LH and merely coincides with nursing-induced releases of prolactin. Observations by Dusza et al. (1990) agree with this theory, reporting that exogenous prolactin administered throughout lactation had no effect on plasma LH or the pre-ovulatory LH surge in sows. This is also supported by experiments where bromocriptine suppressed prolactin in lactating sows without influencing LH concentrations (Mattioli et al., 1986; Farmer et al., 1998), as well as studies where temporary removal of the litter (and subsequent decrease in prolactin) failed to show any relationship with plasma LH (Parvizi et al., 1976; Stevenson et al., 1981). However, reports by Kraetzl et al. (1998) and Van de Wiel et al. (1985) observed consistently lower prolactin levels in sows that spontaneously ovulated during lactation, with the authors suggesting that the lower prolactin levels reduced the inhibition of the GnRH pulse generator. A study by Bevers et al. (1981) supports this theory by demonstrating that suppression of ovarian activity in lactating sows is not due to an inhibitory effect of prolactin at the pituitary level.

Although prolactin's direct role on gonadotropins remains unclear, prolactin's additional roles during lactation may also be relevant. Prolactin is important for the induction and maintenance of LH receptors in luteal cells (Holt et al., 1976). Furthermore, Basini et al. (2014)

showed that the ovary is also a target organ for prolactin activity, suggesting that the hormone has an inhibitory effect in the early phase of follicular development. While incomplete, the current literature suggests that while prolactin may partially account for LH suppression during lactation, it seems likely that the primary LH block comes at the hypothalamic level or higher (Van de Wiel et al., 1985).

Boar Stimulus Value

Pheromones produced by the boar stimulate estrous activity in gilts and sows. Additionally, the presence of a boar enhances the sow's expression of estrus and increases the likelihood of estrus detection by the handler (Langendijk et al., 2000), which has led to the routine use of a boar during estrus detection (Hemsworth et al., 1990). When lactating sows were exposed to a mature boar or to a synthetic boar pheromone (5α-androst-16-en-3-one), the WEI interval was reduced to a similar extent (reviewed by Britt et al., 1985). Despite these potential stimulatory effects of synthetic boar pheromone, Gerritsen et al. (2005) showed that successful estrus detection was less likely to occur when a robotic boar providing visual, auditory, and olfactory (5α-androst-16-en-3-one) cues was compared to the physical presence of a mature boar. Since estrus detection is a prerequisite for rebreeding success, at this time it appears the physical presence of a boar remains an essential ingredient in order to provide the combination of pheromones and non-olfactory stimuli needed.

Multiple factors influence the efficacy and consistency of the boar stimulatory effect; (reviewed by Hughes et al., 1990); in particular, the influences of the individual boar, the degree of contact between boar and females, and the frequency and duration of boar contact must also be considered. In selecting the level of boar contact necessary, it is important to note that while increased stimuli (boar alone vs. boar + back pressure test vs. detection mating area) is more

likely to evoke a standing response, sows have been known to adapt their responsiveness to the highest stimulus level (Langendijk et al., 2000), suggesting that day to day stimulus consistency may be more important than the magnitude provided (Soede et al., 2012).

Beyond estrus detection, boar contact effects on specific endocrine responses in the lactating sow are less understood. Olfactory elements of boar presence may affect the release of hormones and neuropeptides at the hypothalamic-pituitary axis which are known to be important in regulation of LH pulsatility (Booth and Baldwin, 1983). Van de Wiel et al. (1993) reported an increase in LH pulsatility after weaned sows were first exposed to a boar. In Langendijk et al. (2000), boar contact increased the number of ovulatory primiparous sows, yet no changes in follicular growth were observed. Since follicular growth in later stages is dependent on LH pulsatility (Guthrie et al., 1990), boar exposure may have increased LH pulsatility sufficiently to result in ovulation. For sows with short WEI, boar stimulus seems to have little impact. However, sows with low LH pulse frequency after weaning are more likely benefit from the extra LH release triggered by boar presence (as reviewed by Kemp et al., 2005). These sows typically have an extended WEI, but in some sows, boar contact still does not seem to be sufficient to overcome the low LH and these sows will still remain anestrous. Results of Pearce and Pearce (1992) substantiate this as boar stimulation had the greatest effect during periods of seasonal infertility when prolonged WEI commonly occur.

In gilts, boars induce puberty by stimulating a rise in estradiol concentrations (Paterson, 1982). Additionally, tactile stimulation by a boar is associated with release of cortisol in the gilt (Pearce and Hughes, 1987), which increases basal LH secretion (Pearce et al., 1998) and may be associated with the onset of follicular development. The reduced efficacy of the boar effect when fence-line contact is provided without tactile stimulation further suggests that acute cortisol

release may also be important in sows (Hughes et al., 1990; Langendijk et al., 2000). Conversely, pharmacological evidence indicates that long-term elevation of ACTH or cortisol can suppress LH release and estrous behavior (Barb et al., 1982; Turner et al., 1999). The importance of tactile boar cues still appears to be important in genotypes used currently. When nursing the entire litter, Terry et al. (2013) observed lactational estrus in 56% of sows provided daily fence-line boar contact, but Weaver et al. (2014) reported that by using full boar contact in a detection mating area, lactational estrus incidence increased to 67%, despite a shorter lactation length (26 vs. 30 d). These levels of lactational estrus using boar exposure as a stimulant are considerably higher than earlier reports (56 to 67% vs. 0 to 13%; Rowlinson and Bryant, 1982; Newton et al., 1987a). Langendijk et al. (2009) demonstrated that certain sow lines are less responsive to lactational estrus induction strategies than other genetic lines (28 vs. >90%; Langendijk et al., 2007; Gerritsen et al., 2008b). Accordingly, this greater response to boar exposure may be related to genetic selection for short WEI leading to some populations of sows more predisposed to ovulate during lactation.

FOLLICULAR DEVELOPMENT

Classical experiments on follicular growth had to be collected via sequential slaughter, limiting the knowledge gained to morphological changes over time, not allowing for evaluation of patterns of follicular growth within individual sows (reviewed by Lucy 2001). The advent of ovarian ultrasonography revolutionized the study of ovarian function because follicular growth could be evaluated daily on individual animals (Pierson et al., 1988). In monovular species such as cattle, follicle growth prior to ovulation is known to be wave-like, where a cohort of follicles grow in synchrony until one follicle becomes dominant and continues to grow while the other follicles regress at variable intervals (Evans, 2003). The classical view that domestic pigs are

exceptions by not displaying follicular waves during estrous cycles (Evans, 2003) has been challenged by recent ultrasonic observations (Lucy, 2001; Noguchi et al., 2010). When taken together with the inverse relationship between circulating inhibin A and FSH concentrations detected in sows (Noguchi et al., 2010), these observations indicate that follicle growth during the early luteal phase may actually be analogous to that of cattle.

Follicular measurements taken by Noguchi et al. (2010) confirmed that follicles rarely exceed 5 mm in diameter prior to weaning. However, Lucy et al. (1999) described 4 distinct patterns of follicular growth in lactating sows prior to weaning. For a small percentage of lactating sows, follicular growth leads to lactational ovulation because these sows overcome suckling inhibition and regain the ability to mount a pre-ovulatory LH surge before weaning. However, in the absence of an LH surge, cystic follicles can form if estradiol fails to return to basal levels and low progesterone levels persist (Gerritsen et al., 2014). In early-weaned sows (< 14 d; Castagna et al., 2004) or attempts to stimulate ovulation during lactation within 14 d post-farrowing (Langendijk et al., 2009), insufficient pituitary LH pools or deficient feedback from estradiol may also result in failure to mount an LH surge and result in cystic ovaries. A third pattern of pre-weaning development is characterized by the continued presence of small follicles (< 2 mm) that represent general ovarian inactivity. This pattern may occur more frequently in primiparous sows, sows in poor body condition (Prunier and Quesnel, 2000) or heat-stressed (Lucy 2001) sows, and is likely to result in an extended WEI. In the fourth pattern of follicular growth, synchronized waves of follicles can be observed growing and regressing prior to weaning. For these sows, the WEI will vary depending on the stage of follicular development at the time of weaning (Lucy 2001). The aforementioned variation in pre-weaning follicular

development presents challenges for lactational ovulation induction protocols and is important to understand to design an efficient application strategy.

Reviews of lactational ovulation induction strategies (Langendijk et al., 2007, Soede et al., 2009) show that by using multiparous sows of a receptive genotype and initiating boar contact and suckling manipulation beyond 14 d of lactation, normal follicle development and lactational ovulation can occur in 90 to 100% of sows. Given the variable patterns of follicular development during lactation, tightening the variation in the ovulatory response seems to be the greater obstacle. Application of exogenous hormones may help, but their use may be averse to public opinion (Kemp and Soede, 2012a).

METABOLIC STATE OF THE SOW

The primacy of lactation causes partitioning of nutrients toward milk production, which results in a negative energy balance and leads to catabolism of the sow's body fat and protein reserves. In the 1970's and 1980's, feed restriction during lactation chiefly resulted in prolonged WEI, with little impact on ovulation rate or embryo survival (reviewed by Soede and Kemp, 2012). More recent data suggests that genetic selection has made contemporary sows more resilient to the effect of lactational feed restriction on WEI, but effects on ovulation rate and litter size are more severe than previously thought (Kemp and Soede, 2012). In a study by Zak et al. (1997), subsequent reproductive performance in primiparous sows was reduced regardless of whether low feed intake occurred early or late in lactation. Data collected from 15 modern commercial sow farms in Germany and Slovakia showed that subsequent reproductive performance is negatively impacted when sows lose more than 10% of their BW during lactation (Thaker and Bilkei, 2005). Low lactation feed intake causes inhibition of LH pulsatility (Quesnel et al., 1998) as well as impaired follicle quality and maturation (Zak et al., 1997) prior to

weaning. Metabolic hormones such as insulin and IGF-1 require several days after weaning to return to normal levels (van den Brand et al., 2001; Mejia-Guadarrama et al., 2002), and suboptimal concentrations of these hormones may be related to reports of reduced ovulation rate and embryo survival in sows with WEI shorter than 3 d (as reviewed by Quesnel, 2009). This hypothesis is supported by experiments where post-weaning rebreeding was intentionally delayed by using altrenogest (a progesterone analogue) or by skipping the first estrus after weaning. These experiments resulted in increased ovulation rate and/or higher embryo survival (Wellen et al., 2007; Patterson et al., 2008). These metabolic sequelae may also be related to the outcome of a survey of Norwegian sows where lactating sows mated prior to 21 d post-farrowing had poorer subsequent farrowing rates and litter size (Gaustad-Aas, 2004).

Overcoming the lactation-induced negative energy balance may be essential for lactational ovulation to occur; in fact, lactating sows with high feed intake and low BW loss are more likely to spontaneously ovulate (Kraetzl et al., 1998) and are the most responsive to lactational ovulation induction strategies (Petchey and Jolly, 1979; Rowlinson and Bryant, 1982). In conjunction with lactation, the inherent metabolic demands for continued lean tissue deposition make sows nursing their first litter more sensitive to lactation weight losses (Foxcroft et al., 1997), which result in lower lifetime productivity and increased culling rates (Hoving et al., 2011). This so called “second litter syndrome” likely also contributes to the decreased incidence of lactational estrus in primiparous sows (Stevenson and Davis, 1984a; Soede et al., 2012). Thus, any successful lactational estrus induction strategy must be able to mitigate, or at minimum take into account, the challenges associated with first parity sows and sows experiencing excessive BW losses during lactation.

SEASONAL INFERTILITY

In pigs, decreased reproductive performance during summer and autumn is common (Xue et al., 1994; Auvigne et al., 2010), but limited information is available regarding seasonal effects on the incidence of lactational ovulation. Although domesticated pigs are typically regarded as non-seasonal breeders, the wild boar primarily breeds during winter (Mauget 1982). Seasonal infertility in domesticated pigs may be a relic of the seasonal breeding pattern present prior to domestication (Peltoniemi et al., 2001). In many species, prolactin acts as a luteotrophin or a luteostatin, mediating seasonal changes in reproduction (Curlewis, 1992). Seasonal fluctuations in prolactin occur in the domesticated pig, but to a lesser extent than in wild boar and seasonal breeders such as sheep (Ravault et al., 1982). Therefore, prolactin does not appear to be the primary cause of seasonal variations in pig fertility.

Changes in temperature likely play a significant role. When ambient temperatures exceed the evaporative critical temperature of the sow (22˚C; Quiniou and Noblet, 1999), sharp decreases in lactation feed intake are consistently observed (-215 to 430 g-1˙d-1/˚C; reviewed by Gourdine et al., 2006). This decrease in sow feed intake results in mobilization of body protein and fat reserves, negatively impacting sow fertility. Due to limited body reserves at farrowing, primiparous sows are the most susceptible to the negative effects of high ambient temperature on reproductive performance (Hughes, 1998), usually typified by prolonged WEI intervals (Aumaitre et al., 1976; Britt and Levis, 1983; Xue et al., 1994). This is supported by an evaluation of herd records from 42 commercial U.S. swine farms (Xue et al., 1994), who reported decreased farrowing rates and extended WEI for sows bred during summer and early autumn, particularly in first parity sows. During summer heat stress conditions, increased culling

rates and irregular returns reflected increased rates of abortion and failed conception (Xue et al., 1994).

Nevertheless, high ambient temperatures alone do not explain seasonal fluctuations in reproduction. In a five-yr study of 266 farms in four regions of France, similar levels of seasonal infertility, defined as the ratio of sows found pregnant at 4 wk relative to the number of sows mated, occurred annually across all regions regardless of the number of hot days per year (Auvigne et al., 2010). Since year-to-year variation in seasonal temperature fluctuation did not explain differences in seasonal infertility levels, it was alternatively hypothesized that photoperiod changes may also contribute to seasonal infertility (Auvigne et al., 2010). This is consistent with Paterson and Pierce (1990), who reported short-day lighting regimens resulted in earlier attainment of puberty in gilts compared to long-day lighting patterns, and also agrees with observations that sows weaned under a long photoperiod have an extended WEI (Prunier et al., 1994).

The negative impact of breeding during summer and fall in weaned sows seems to correspond with the variation in lactational estrus incidence in previous reports. In an intermittent suckling experiment conducted during winter (Stevenson and Davis, 1984a), 45 and 76% of primiparous and multiparous sows expressed lactational estrus; however, similar experiments conducted during May and August yielded no first parity sows and only a small number of multiparous sows in estrus prior to weaning (Newton et al., 1987a; Newton et al., 1987b). Similar seasonal fluctuations in lactational estrus occurrence have been noted when sows are grouped during lactation in the presence of a boar (Petchey and Jolly, 1979; Hulten et al., 2006). The consistency of reduced lactational ovulation occurrence during summer and autumn indicates that seasonal inhibition of gonadotropins may also be involved. Some management

interventions may help such as keeping sows under conditions of decreasing light from May to August (Claus et al., 1984) and providing drip or snout coolers (McGlone et al., 1988). While the primacy of contributing influences remain unclear, seasonal changes appear to be an important factor affecting the variation in response to lactational estrus induction protocols and must be addressed to achieve consistent estrus responses.

CONCURRENT LACTATION AND PREGNANCY

In situations where rebreeding occurs during lactation, an important consideration is the impact of continued lactation during pregnancy on subsequent reproductive performance. Gaustad-Aas et al. (2004) reported reduced farrowing rates and litter size when lactating sows were inseminated within 21 d post-farrowing, but these reductions were similar to those of early-weaned sows inseminated before 21 d and no differences were observed between lactating and non-lactating sows if mating occurred after 21 d postpartum. Gerritsen et al. (2008b) reported lower pre- and post-ovulatory progesterone concentrations when sows were inseminated during lactation and continued lactating during pregnancy. This is supported by a follow-up study (Gerritsen et al., 2009) where sows that were weaned immediately after lactational ovulation had higher progesterone and tended to have a higher pregnancy rate compared to sows that continued intermittently suckling until d 20 of pregnancy. Furthermore, a recent report by van der Peet-Schwering et al. (2015) emphasizes the importance of maintaining high feed intake if sows are concurrently gestating and lactating. If high feeding levels cannot be maintained, greater sow body weight and back fat losses are likely to occur and may contribute to reduce embryo survival in the subsequent litter. Nevertheless, a review by Kemp and Soede (2012a) concluded that continued intermittent suckling during pregnancy has minimal effects on embryo survival and farrowing rate as long as the litter separation is at least 10 to 12 h/d and the intermittent suckling

does not extend beyond the first week after breeding. In Terry et al. (2014), multiparous sows mated while lactating a split-weaned litter had reduced subsequent litter size versus split-weaned sows mated post-weaning; yet surprisingly, the subsequent fertility of primiparous split-weaned sows mated during lactation was not impacted. The authors speculated that these effects may be confounded by the fact that sows split-weaned but remaining anestrus until after weaning had improved subsequent litter size versus those conventionally weaned. Moreover, sows bred during lactation were grouped immediately after weaning, experiencing mixing stress at 8 to 9 d post-mating, a time which may reduce pregnancy rates in recently bred sows (Knox et al., 2014). Further support for minimal impact of concurrent lactation and pregnancy can be drawn from a report from an organic system allowing extended lactation lengths (56 d; Kongsted et al., 2009). In that study, 84% of grouped sows expressed lactational estrus and although sows continued to be nursed for 8 to 16 d, there was no apparent impact on subsequent litter size (13.6 born live). Additional work is needed to continue to clarify management considerations for concurrently lactating and gestating sows in order to minimize effects on fecundity. Based on the available literature, recommendations provided by Soede et al. (2012) to ensure that lactational mating occurs at beyond 21 d of lactation and minimize simultaneous lactation and pregnancy to less than 7 d currently seem sufficient to avoid major negative impacts.

LACTATIONAL ESTRUS INDUCTION STRATEGIES

Dating back to the early 20th century (Robeson, 1918), various techniques have been tested to induce lactational estrus. These include various presentations of boar stimuli, temporary separation of sow and litter (intermittent suckling), partial weaning of the litter (split-weaning), grouping of lactating sows, and exogenous hormone treatments (reviewed by Alonso-Spilsbury et al., 2004). Typically, researchers have combined multiple factors with variable results.

Although many of the applied techniques have been further refined, since 2000, greater success rates have been observed (22 to 100%; reviewed by Terry et al., 2014). Emphasis on genetic selection has yielded hyperprolific sow lines able to return to estrus shortly after weaning, which may have contributed to current sow lines that are more predisposed to lactational ovulation. A review of recent and classical experiments evaluating each factor will be presented below.

Boar Contact

Provision of boar exposure to lactating sows has been accomplished in several ways. These include fence-line nose-to-nose contact (Mota et al., 2002; Downing et al., 2012; Terry et al., 2013), full contact for a limited period each day (Stevenson and Davis, 1984a; Newton et al., 1987a; Costa and Varley, 1995; Kirkwood and Thacker, 1998; Downing et al., 2007), continuous boar presence after grouping sows in lactation (Rowlinson et al., 1975; Kongsted and Hermansen, 2009), or the temporary removal of sows to a detection mating area (van Wettere et al., 2013; Terry et al., 2014; Weaver et al., 2014;). A detection mating area is a pen surrounded by 4 to 6 crated boars designed to maximize boar stimuli (Jongman et al., 1996). These different presentations likely influence the occurrence of lactational ovulation (Kemp et al., 2005), but confounding factors such as altered suckling, exogenous hormone treatments, the timing of initial exposure, and interactions between these variables prevent definitive conclusions.

With few exceptions (Rowlinson and Bryant, 1975; Stolba et al., 1990), early efforts to use boar contact alone to stimulate lactational estrus in lactating sows had limited success (0 to 13%; Rowlinson and Bryant, 1982; Walton 1986; Newton et al., 1987a; Henderson and Stolba, 1989). By combining boar exposure with additional stimuli, other experiments during the 1980's and 1990's typically failed to isolate boar response in their experimental design and generally had inconsistent results irrespective of additional stimuli provided. In stark contrast to earlier

reports, recent studies show greater response to boar exposure alone (55 to 67%; van Wettere et al., 2013; Terry et al., 2013; Terry et al., 2014; Weaver et al., 2014). As both fence-line and full boar contact were used in earlier studies as well as in more recent experiments, it is unlikely that differences in degree of boar stimulus were responsible for the differences in lactational ovulation observed in recent versus historical efforts. Genetic selection against prolonged WEI may be linked to the increased estrus response, as contemporary dam lines are more resilient, with negligible effects on WEI even when nutrients are restricted (reviewed by Kemp and Soede, 2012a).

Despite the increased rate of boar-stimulated lactational estrus in recent studies, the reproductive rates remain lower than sows conventionally mated post-weaning. Accordingly, it appears that incorporation of other factors (e.g. altered suckling or exogenous hormones) are necessary to elicit lactational estrus responses comparable to conventional weaning. Nevertheless, these studies provide definitive proof that provision of boar component stimuli plays a role in lactational estrus stimulation. Considering the essential role boars also play in estrus detection (Kemp et al., 2005), the use of some level of boar stimulation is likely to be included in lactational estrus induction protocols. Based on the literature available, at a minimum, fence-line boar exposure for 15 min/d appears to be adequate to invoke the boar stimulatory response. Future research should consider use of remote-controlled boar carts or small mature boars on boar stimulatory effects. Known for their early sexual maturity (Kanematsu et al., 2006), increased pituitary activity (Wise et al., 1996) and good disposition, Meishan boars may be a safe, effective way to deliver the boar stimulus to the lactating sow.

Intermittent Suckling (IS)

A consistent feature of successful lactational ovulation is the reduction of the suckling stimulus of the piglets, thereby reducing EOP-mediated suppression of LH secretion and resulting in follicular development. One way to reduce suckling is to provide temporary separation of sows and their piglets. This approach is most commonly referred to as intermittent suckling (IS), but has also been called reduced suckling, limited suckling, or interrupted suckling. The earliest known report delivers a surprisingly prescient recommendation, stating that "Lactating sows may be brought into heat by the simple expedient of separating the young from their mothers for four or five nights, allowing the pigs to suckle only during the day" (Robeson, 1918). However, IS was not seriously reexamined until the second half of the 20th century, when Smith (1961) endeavored to increase energetic efficiency in the lactating sow and Crighton (1970a, 1970b) combined IS with injections of pregnant mare serum gonadotropin in an effort to reduce the farrowing interval, as typical lactations were a minimum of 42 d at the time. Provision of 12 h of IS per day in these experiments resulted in high incidence of lactational estrus (79 to 93%) and led to continued refinement of IS techniques. Several experiments indicate that IS intervals as short as 3 to 6 h, when combined with boar exposure, may be sufficient to induce high rates of lactational estrus in multiparous sows, but IS for this short a period seems inadequate for primiparous sows (Stevenson and Davis, 1984a; Stevenson and Davis, 1984b; Newton et al., 1987a; Newton et al., 1987b). However, other reports indicate that IS, even for 12 h in multiparous sows resulted in only isolated cases of lactational estrus (Henderson and Hughes, 1984; Costa and Varley, 1995).

More recently, Langendijk et al. (2007) and Gerritsen et al. (2008a) reviewed the optimal presentation of IS and the effects on sow reproductive performance. They reported that up to

90% of sows are likely to show lactational estrus if the following conditions are met: 1) IS should not be initiated until d 18 postpartum, 2) IS should last for at least 10 h/d, 3) during IS, sows should be housed out of sight and sound of piglets, and 4) some form of boar contact should be provided. These recommendations are consistent with results of some recent experiments (Downing et al., 2011; Downing et al., 2012); but Soede et al. (2012), following these recommendations, found only 23% of primiparous and 68% of multiparous sows in estrus during lactation. This variation may be related to disparity in the amount of primiparous sows across IS experiments as well as variation in genotypes, which Langendijk et al. (2009) demonstrated is an important factor for successful induction of lactational estrus.

It is worth noting that sows responding to IS treatment typically do so in a synchronous fashion approximately 4 to 5 d from the onset of treatment (reviewed by Soede and Kemp, 2015), and extending the IS treatment period from 7 to 14 d did not change the lactational estrus response (Soede et al., 2012). Shorter IS treatment durations (2 or 3 d) could be sufficient to induce a fertile estrus, but this has not been tested. Interestingly, non-responding sows consistently show a 'normal' WEI interval, supporting earlier claims that the lactational estrus response to IS regimens is an 'all or none' phenomenon (Stevenson and Davis, 1984). However, when IS starts too early in lactation (<14 d), some of the sows showing lactational estrus may develop cystic ovaries and fail to ovulate (Langendijk et al., 2009; Downing et al., 2011). As discussed earlier, the development of cystic ovaries is likely associated with insufficient accumulation of LH pools to mount a pre-ovulatory LH surge (Gerritsen et al., 2014).

Consequences of lactational mating after IS on subsequent fertility deserve additional attention. Recent IS studies (reviewed by Soede et al., 2015) indicate reduced pregnancy rate and embryo survival as well as impaired embryo development if IS-induced ovulation occurs as early

as 19-21 d post-partum and if IS continues for 20 d beyond ovulation, possibly related to reduced progesterone concentrations in these sows (Gerritsen et al., 2008b). However, per earlier recommendations, if insemination occurs beyond 21 d after farrowing and intermittent suckling does not extend beyond 9 d post-mating, no negative effects have been reported (Gaustad-Aas et al., 2004; Downing et al., 2012; Soede et al., 2012). Nevertheless, the variation in the number of sows showing estrus remains a limitation, but focus on use in responsive genotypes and excluding first parity sows should ensure good reproductive output (Kemp and Soede, 2012a). Combining an IS regimen with exogenous hormone treatments may also aid in synchronizing the response.

Litter Performance and IS

Consequences of IS on piglet performance are important. Conventional weaning takes place abruptly and places numerous stressors on the newly weaned pig simultaneously. These include transport, a new housing environment and mixing with unfamiliar pigs. One of the most important changes is the transition from a milk diet to a solid, non-milk diet. Creep feed is often provided during late lactation in an effort to familiarize piglets with solid food prior to weaning. Pigs that consume creep feed prior to weaning have higher feed intake (Bruininx et al., 2002; Sulabo et al., 2010), increased intestinal absorption (Kuller et al., 2007a), and reduced villous atrophy (van Beers-Schreurs et al., 1998) in the early post-weaning period. However, creep feed intake is generally low during conventional lactation (as reviewed by Langendijk et al., 2007) and there is considerable variation in creep feed consumption between and within litters (Pajor et al., 1991; Kuller et al., 2004).

Intermittent suckling provides a period of separation between the sow and piglet prior to complete weaning, more closely mimicking the natural weaning process where sows gradually

reduce suckling frequency and time spent with piglets (Rantzer et al., 1995). This temporal separation stimulates pre-weaning creep intake but does not appear to reduce the between litter variation in feed intake (Kuller et al., 2004; Kuller et al., 2007b). A higher percentage of litters subjected to IS reached a cumulative pre-weaning intake of more than 600 g per pig (Castellano et al. 2014; Kuller et al., 2004), a threshold identified by English et al. (1980) as the amount necessary to improve growth during the early post-weaning period. Nevertheless, the reduced suckling opportunities during an IS regimen will reduce piglet growth prior to weaning (Thompson et al., 1981; Henderson and Hughes, 1984; Kuller et al., 2004; Berkeveld et al., 2007a), and the growth suppression is more severe with increased separation duration (Berkeveld et al., 2007a; Downing et al., 2012). Although minimizing the depression in piglet growth prior to weaning is preferred, it appears that less than 8 h of IS per day may not stimulate creep feed intake to an extent that will improve post-weaning intake and growth (Millet et al., 2007). Considering also that at least 10 h of IS per day is needed to elicit high levels lactational ovulation (reviewed by Langendijk et al., 2007; Gerritsen et al., 2008a), an IS period of 10 to 14 h per day is recommended.

The effects of initiation time and IS treatment duration have also been studied. Berkeveld et al. (2009) found that extending the IS regimen from 7 to 14 d provided no additional benefit to piglet performance, but improvements in post-weaning growth for IS pigs were more profound when a 7 d IS regimen occurred alongside an extended lactation (33 vs. 26 d). Recently, Downing et al. (2012) reported that only 3 d of overnight IS can stimulate lactational ovulation in a high percentage of sows. Moreover, a 3 d IS duration lessened the negative impact of IS on pre-weaning piglet growth. Kemp and Soede (2012) posited that shorter IS durations (2 to 3 d) would require less labor and are more likely to be adopted commercially; however, it is

important to consider that expected post-weaning growth benefits may be compromised if the entire litter is once again placed back on the sow and allowed to continuously suckle until weaning.

When an IS regimen is applied for 12 h/d for 7 d, IS pigs will be lighter at weaning, but will experience a post-weaning growth check only 25 to 30% as severe as pigs conventionally weaned. Generally, pigs subjected to IS regimens are similar in BW to conventionally-weaned pigs by 7 d post-weaning (Kuller et al., 2004; Berkeveld et al., 2007a; Berkeveld et al., 2009) and finishing ADFI and ADG are not affected (Kuller et al., 2007b). Thus, Langendijk et al. (2007) concluded that IS regimens can result in a more gradual adaptation to the post-weaning period and thereby reduce the risk of post-weaning diarrhea, but have little impact on long term growth performance.

One of the current limitations for IS implementation is the additional labor required at the farm level. Now that the effects of various IS protocols on piglet performance and sow fertility are well-understood, research attention should focus on development of equipment and presentations to easily implement IS regimens on farms. Day- versus night-time separation may also have an impact on piglet and sow behavior and performance (Berkeveld et al., 2007b), but information is currently limited on this aspect. A final area receiving little attention is the effect of IS on milk yield. Thiel et al. (2005) showed that lactation capabilities can be rescued when piglets are removed for 24 h and then placed back on the sow; however, the milk output of these sows was reduced by 15 to 20%. Intermittent-suckling separation periods are typically shorter (8 to 16 h), but it is unknown whether these shorter separations impact milk yield. Effects on milk yield for sows of different parities should also be studied.

Split Weaning (SW)

Also called fractionated weaning, SW refers to permanent removal of a portion of the litter (typically the heaviest piglets) prior to weaning the remaining lightweight pigs a few days later. Split weaning has also been used to elicit lactational ovulation by reducing the suckling stimulus. Prolonged and variable WEI were common in the 1970's and 1980's (reviewed by Quesnel, 2009) and SW was initially used to decrease WEI and better synchronize post-weaning estrus (Stevenson and Britt, 1981; Cox et al., 1983). Permanently removing a portion of the litter reduces the lactation demand for nutrients and reduces the catabolism of sow energy and protein stores. Lactation-induced negative energy balance is considered a primary cause for the extended WEI typically observed in primiparous sows (Hoving et al., 2011) and in sows that lose >10% of their BW during lactation (Thaker and Bilkei, 2005).

Split-weaning all but 6 piglets from d 21 to 28 (weaning) reduced lactation BW and back fat loss in first and second parity sows, narrowing the variation in WEI and improving WEI and subsequent farrowing rate in second parity sows (Vesseur et al., 1997). Given that SW had a greater effect in second parity sows, the authors concluded that alleviating the negative energy balance during lactation was the primary reason for improved reproductive performance. Primiparous sows have lower lactation feed intake and body reserves than older sows and may have been unable to overcome their negative energy balance and restore follicular development prior to weaning. However, endocrine responses were not measured by Vesseur et al. (1997). Zak et al. (2008) maintained an equivalent energy balance across treatments, and found that reducing litter size to four nursing piglets caused an initial, but transient increase in LH concentration and pulse frequency, and an earlier resumption of ovarian activity as indicated by more follicles larger than 3 mm by 1 d post-weaning (Zak et al., 2008). However, the circulating

IGF-1 was elevated for SW sows on d 21 and the authors suggested that energy balance may not be the only indicator of metabolic status to consider.

Decreased prolactin levels after implementing SW may have also played a role (Degenstein et al., 2006), as prolactin has previously been associated with suppression of GnRH prior to weaning (Van de Wiel et al., 1985). Since the LH responses were transient, but sufficient for resumption of follicular development, Zak et al. (2008) postulated that as little as 3 d of SW may be sufficient. However, Tarocco et al. (2000) only detected a benefit to reproductive performance when the SW protocol was at least 6 to 7 d, whereas a 5 d SW regimen elicited no effects. This contradiction is indicative of the variable efficacy reported using SW treatments with suggested SW durations varying from 2 to 3 d (Stevenson and Britt, 1981; Cox et al., 1983; Zak et al., 2008), 5 d (Matte et al., 1992), or 7 d (Vesseur et al., 1997; Tarocco et al., 2000) and in some instances no response was observed (Gilbertson et al., 1989; Rojkittikhun et al., 1990). Reasons for the variation in response are likely multifaceted and these experiments differed in the number of piglets remaining and the duration of the SW as well as the parity, metabolic status and genotype of the sows. Reviews by Matte et al. (1992) and Soede et al. (2009) conclude that the most important variable is the number of pigs remaining during the last days of lactation. The largest reduction in WEI was when only 3 pigs continued to nurse. Overall, SW appears to be most effective for sows that would otherwise have an extended WEI.

Until recently, using SW to induce estrus during lactation had not been considered (Terry et al., 2013). When SW was initiated at d 18 postpartum and continued until weaning at d 30, SW plus fence-line boar exposure elicited a high rate of lactational estrus (83 to 95%) regardless of whether 3, 5 or 7 pigs were removed from an initial litter of 10. However, removing only 3 piglets at d 18 numerically decreased the incidence of lactational estrus and decreased

conception rate compared to the controls. Interestingly, it seems that for almost all sows with 5 or 7 pigs removed, lactational estrus was observed by d 24. The lack of a control treatment limits interpretation of subsequent reproductive performance in this study, but in a follow-up experiment (Terry et al., 2014), SW sows with 7 piglets remaining showed lactational estrus 89 and 61% of the time for primi- and multi-parous sows, respectively, but decreased farrowing rate (75 vs. 83%) and NBA (10.4 vs. 11.1) were reported when sows were mated during lactation versus post-weaning. In that experiment, treatments were applied in a commercial environment with 299 sows per treatment. Although the provision of full boar contact in a detection mating area may have also contributed to the high lactational estrus rates, this data suggests that SW as few as 3 piglets can elicit lactational estrus at high rates that are comparable to IS treatments. Similar to observations in IS studies, responsive sows typically express estrus within 4 to 5 d, primiparous sows are less likely to respond and the provision of adequate boar exposure remains important. Moreover, comparable to IS experiments, sows not expressing lactational ovulation seem to show a normal WEI compared to sows conventionally weaned (Terry et al., 2014). While the poorer subsequent reproductive performance observed for SW sows mated in lactation is a concern, the results in Terry et al. (2014) may have been confounded by the fact that sows mated in lactation were mixed at d 8 to 9 post-insemination. Also, sows that were SW but did not show lactational estrus had numerically higher farrowing rates (94 vs. 83%) and NBA (11.6 vs. 11.2) compared to control sows. This suggests that follicular growth was still improved by SW in the non-responsive sows (supported by van Leeuwen et al., 2012) and their improved fertility may have artificially inflated the subsequent reproductive performance of sows mated post-weaning. Overall, the use of SW to induce lactational estrus appears promising, but effects on sow fertility need to be clarified. The level of piglet reduction needs to be further defined,

evaluating both the number or percentage of pigs removed as well as the number remaining on the sow. Further information on the impacts of timing and level of boar exposure on efficacy of lactational estrus induction are also needed to develop commercial SW protocols.

Litter Performance and SW

The effects of split-weaning on litter performance are well-characterized. A review by Matte et al. (1992) described SW as 'not detrimental to the piglets'. During the SW period, lightweight pigs allowed to continue to nurse consistently outperform their weaned heavyweight counterparts. This is likely due to additional access to milk and less competition and exacerbated by the simultaneous post-weaning growth check experienced by the weaned heavyweight pigs (Cox et al., 1983; English et al., 1987; Mahan 1993; Pluske and Williams, 1996; Vesseur et al., 1997; Terry et al., 2013; Terry et al., 2014). If SW occurs in situations with lactation lengths beyond 28 d, greater creep intake prior to weaning may buffer the post-weaning growth check of the SW heavyweight piglets (Matte and Close, 1987; Gilbertson et al., 1989). Regardless, the lightweight piglets do not maintain this growth rate advantage for very long beyond weaning. Unlike intermittently suckled pigs, lightweight piglets in SW regimens experience a normal post-weaning growth check and typically remain lighter than the earlier weaned pigs by 2 wk post-weaning (reviewed by Matte et al., 1992) and through the end of the nursery phase (Pluske, 1996) and grow-finish period (Mahan 1993). While the growth benefits of lightweight SW pigs appear to be transitory, they may impact post-weaning morbidity and mortality. Vesseur et al. (1997) reported a tendency for reduced morbidity (7.8 vs. 14.2%) and numerically lower mortality (2.1 vs 3.7%) in SW pigs versus pigs conventionally weaned at 28 d, but in other reports this data is not available. Unfortunately, earlier reports also gave little attention to the variation in piglet weights among pigs subjected to SW regimens. Mahan (1993) noted that

lightweight pigs nursing for an additional 7 d in a SW treatment reached market 2 d sooner than conventionally weaned lightweight pigs, but the effect was not significant. A current emphasis of the US swine industry is to reduce variation in finishing pig BW (Tokach, 2004). Therefore, research in SW should address its impact on variation in BW at market.

Intriguingly, Van der Heyde and Lievens (1982) reported that female pigs continuing to nurse in SW litters from d 12 to 40 of lactation had enhanced reproductive capacity later in life. Female pigs raised in small litters (≤ 6) have been shown to have greater litter sizes than those raised in large litters (Nelson and Robinson, 1976; Van der Steen, 1985; Kirkpatrick and Rutledge, 1988), but other reports have shown no maternal effects on nursing litter size (Deligeorgis et al., 1985; Stewart and Diekman, 1989). It is unlikely that SW should have a major impact on female piglet reproductive capacity since it typically occurs for a short period of time at the end of lactation, but future research should investigate this potential phenomenon, particularly if heavy weight females are SW.

Grouping Lactating Sows

Commercial use of group housing for lactating sows is limited; however, bans on individual housing during gestation in the European Union, Australia, and Scandinavia have resulted in renewed interest in such systems. These restrictions may also be accompanied by extended lactation lengths (> 21 d; Gaustad-Aas et al., 2004), when sows are more likely to escape the suckling-induced inhibition of LH release (Bevers et al., 1981; Varley and Foxcroft, 1990) and spontaneously ovulate (McDonald et al., 2015). Moreover, the presence of foreign piglets may impact the nursing behavior of the sow and thereby also affect the incidence of lactational ovulation (Kemp and Soede, 2012b). The increased risk of lactational ovulation in group-housed lactating sows results in more variable and longer WEI if sows are not inseminated

during lactation (reviewed by van Nieuwamerongen et al., 2014). Sundry different approaches of group lactation housing have been used and comparisons between them are beyond the scope of the present review. However, the increased risk of spontaneous ovulation makes batch management of sow groups problematic (Einarsson et al., 2014). As a result, lactational ovulation induction protocols have been tested, with varying results. Hulten et al. (2006) reported poor synchrony of onset of lactational estrus when sows lactated for 49 d, but when Kongsted and Hermansen (2009) introduced a boar after 35 d, all sows showed lactational estrus, with 84% responding within 7 d. Moreover, provision of enriched social environments (small sow groups, full boar contact, outdoor access and bedding) increase the likelihood of lactational estrus (Stolba et al., 1990). Thus, opportunities exist to stimulate and synchronize lactational estrus in group-housed sows. Provision of a boar has been common in past attempts to stimulate lactational estrus but the use of IS or SW in group lactation has not been tested. Altered suckling such as IS or SW may assist in synchronizing the response and if initiated after d 19 of lactation and not continuing beyond 7 d after lactational mating, could result in similar reproductive performance (Soede et al., 2012).

Application of Exogenous Hormones

Induction of lactational ovulation via targeted administration of exogenous hormones has been attempted in several ways. Injections of estrogens, exogenous gonadotropins, GnRH agonists and opioid peptides have been applied at various stages of lactation, with varying results. Since combinations of methods more palatable to consumers such as altered suckling and boar exposure can now elicit high rates of lactational ovulation, hormone treatments have received less attention in recent years. Nevertheless, exogenous hormone treatments may provide

a means to synchronize lactational ovulatory responses, a current limitation to the development of practical, efficacious induction protocols for pig producers.

Estrogens: Estradiol benzoate (EB) has also been attempted as a means to elicit an LH surge and ovulation in lactating sows (Cox et al., 1988; Sesti and Britt, 1993). Cox et al. (1988) observed low lactational estrus rates (1 of 4 sows) when EB was applied in the second week of lactation, but were much higher (8 of 9 sows) when EB was administered in the third or fourth week. However, only one of the sows in estrus ovulated in response to treatment. Sesti and Britt (1993) reported similarly high rates of lactational estrus, with 95% of sows in estrus after EB treatment; however, sows generally failed to mount a sufficient pre-ovulatory LH surge and ovulate in response to treatment. These results show that although EB can successful elicit a behavioral estrus during lactation, suckling-induced suppression of LH remains strong enough to prevent ovulation in these sows.

Exogenous Gonadotropins: Cole and Hughes (1946) were the first to attempt to induce estrus in lactating sows using injections of gonadotropins. Using pregnant mare serum gonadotropin (PMSG), Cole and Hughes (1946) stimulated lactational estrus in 26 of 27 sows with a 95% farrowing rate. While these results were promising, the PMSG treatment was applied between d 39 and 68 of lactation, and sows may have been more receptive due to diminished suppression of LH. Later work with injections of PMSG alone have been less encouraging. Heitman and Cole (1956) and Crighton (1970b) both reported 80% of sows showed lactational estrus, but only 68 and 64% of those sows farrowed after being lactationally-mated, respectively. The response to PMSG seems to be similar in modern sows as well, as Kirkwood et al. (1998) reported 85% of treated sows were in estrus within 7 d of treatment on d 28 of lactation, but farrowing rate was again poorer than untreated sows (65 vs. 96%). In a review of studies using

PMSG in lactating sows, sows lactationally-mated after at least a 21 d lactation were more likely to maintain pregnancy to farrowing (Britt et al., 1985). Subsequent reproductive performance was also improved by using PMSG in tandem with a reduced suckling regimen (Crighton 1970a), prostaglandin F2α (Hausler et al., 1980), or human chorionic gonadotropin (hCG; Hausler et al., 1980; Hodson et al., 1981).

Recent experiments using gonadotropins to stimulate lactation estrus in sows have administered injections of 400 IU equine chorionic gonadotropin (eCG) and 200 IU of hCG (PG600; Merck Animal Health, Madison, NJ). High rates of lactational ovulation (80 to 95%) have been reported in recent Australian work where multiparous sows were given PG600 in conjunction with at least 3 d of a 16 h IS and daily fence-line boar exposure (Downing et al., 2009, 2011, 2012). However, lactational estrus rates and subsequent farrowing rates were poorer if treatments started before d 16 of lactation, the IS period was 8 versus 16 h, or when primiparous sows were used. Interestingly, only 10 of 21 sows ovulated during lactation when PG600 was combined with an 8 d SW regimen (Kirkwood et al., 2013). It is unclear why SW sows failed to respond as frequently compared to similar induction protocols since they simply differed in method of suckling reduction used (IS vs. SW; Downing et al., 2009; Downing et al. 2011; Downing et al. 2012). Perhaps differences in genotype, season, or yet unexplained mechanisms may be involved to this variation, as SW litters were reduced to 5 or 6 pigs in Kirkwood et al. (2013) and in other SW studies this was enough suckling reduction to induce higher rates of lactational ovulation even when gonadotropins were not used (75 to 83%; Terry et al., 2013, 2014). Slight differences in the sequence of methods used to induce lactational estrus are likely to account for some of the observed variation, as Costa and Varley (1995) reported very low rates of estrus in lactating sows given PG600, boar exposure, and a 3 to 12 h IS period.

Collectively, these experiments indicate that exogenous gonadotropins can be an important component of successful lactational estrus induction protocols, but when used independently or in inadequately-structured protocols, reproductive performance will be reduced.

GnRH Agonists: One method to elicit ovulation during lactation is via regular administration of GnRH (reviewed by Britt et al., 1985). In studies where sows were given hourly intravenous infusions (1.5 μg/hr) of GnRH beginning at d 24 of lactation or beyond, lactational estrus was initiated in 100% of sows within 84 to 123 h (Cox and Britt, 1982; Ramirez et al., 1985; Armstrong and Britt, 1985). If GnRH infusions began early in lactation (d 13 to 17) or if higher dosages of GnRH were administered, lactational estrus responses decreased to around 50% of sows (reviewed by Britt et al., 1985). Nonetheless, pulsatile GnRH, administered appropriately appears to promote follicular development and result in estrus and normal ovulation rates comparable to sows conventionally-mated post-weaning (Armstrong and Britt, 1985). These experiments were useful in acquiring a better understanding of the endocrine responses controlling resumption of ovarian activity in lactating sows, but the impracticality of repeated injections of GnRH limits their use in lactational estrus induction. Since those studies were conducted, other GnRH agonists such as buserelin, goserelin, leuprorelin, nafarelin, and triptorelin have been developed and widely applied in human reproductive therapies, but are not widely used in pig production in part due to cost and limited regulatory approval (Brussow et al., 2007). However, an intravaginal gel delivery for a GnRH agonist (triptorelin) has been recently approved for use in the United States. Intended to synchronize ovulation in weaned sows, this delivery method offers practical advantages for GnRH-mediated ovulation induction compared to repeated injections (Stewart et al., 2010; Knox et al., 2011), and could also be tested as a means to synchronize lactational estrus induction protocols. Mature ovarian follicles at the time

of treatment are necessary for successful ovulation induction (Knox et al., 2011). Suckling reduction to induce follicle growth could theoretically be used to provide follicles that would be ovulated by exogenous GnRH.

Opioid Peptides: Experiments conducted where opioid antagonists were administered to lactating sows have demonstrated that the suckling-induced release of EOP suppresses basal LH and LH pulsatility during lactation (Barb et al., 1986; Mattioli et al., 1986; Armstrong et al., 1988). If naloxone, an opioid antagonist, is administered beyond the first 10 d post-partum, LH concentrations increase and prolactin concentrations decrease (De Rensis et al., 1993). However, FSH levels are unaffected by opioid antagonists and follicles remain inactive. Therefore, opioid antagonists are unlikely to be used for lactational estrus induction unless combined with other elements which can stimulate ovarian activity.

CONCLUSIONS

In most current swine farm designs and management practices, initiation of the next pregnancy is impossible during lactation. Yet, selection for reduced wean-to-estrus intervals in contemporary sow lines and the gradual progression toward longer lactation lengths may enable producers to reassess the opportunity to integrate re-mating into the lactation period. Conjointly, adverse public opinion toward sow confinement may force producers to modify sow housing systems, in turn making breeding in lactation a realistic approach to maintain the high fertility and reproductive rates routine in today's modern swine farms. Appropriately implemented, mating in lactation can decrease sow non-productive days and may offer some benefit to the suckling litter.

Early efforts to induce ovulation in lactation elicited varying results, which were likely due to the wide range of protocols implemented and exacerbated by a less responsive lactating

sow than at present. The hyperprolific sow lines used today are more resilient to feed restriction and have shorter wean-to-estrus intervals, even occasionally escaping the suckling-induced suppression of LH and spontaneously ovulating. Accordingly, recent attempts to induce lactational ovulation have been more successful. In particular, optimal responses have been observed when suckling reduction, such as intermittent-suckling or split-weaning, is combined with daily boar exposure and, at times, the administration of exogenous gonadotropins. Applied properly, these induction strategies can elicit lactational ovulation in excess of 90% of sows with no detriment to subsequent reproductive performance or litter growth parameters. High success rates are also likely in group lactation systems and operations with longer lactation lengths. However, considerable variation in response still exists. If sows are bred prior to d 21 of lactation or simultaneously gestate and lactate beyond 7 to 10 d, subsequent fertility will suffer. Moreover, lactational ovulation is less likely to occur in less-receptive genotypes, primiparous sows, and sows with excessive body weight loss or low feed intake during lactation.

Research needs to shift towards addressing the variation in sow response and focus on developing ergonomic, yet efficacious methods which can be easily implemented on-farm. The variability in lactating sow ovulatory responses might be reduced pharmacologically, by targeted application of GnRH agonists or the short-term use of a progesterone analogue; however, their use may be questioned by consumers. Further characterization of follicle development in lactating sows may also aid refinement of induction protocols to diminish variability. Shortening IS periods (2 to 3 d) or SW less heavyweight pigs prior to weaning could make implementation of reduced suckling methods less labor intensive. Altering farrowing accommodation to facilitate suckling reduction should also be tested. Another important research area is distinguishing the magnitude of boar exposure necessary. Provision of fence-line boar exposure within the

farrowing area seems more feasible than sow removal to a detection mating area, and future research should consider using boar carts and/or Meishan boars to deliver boar component stimuli. The effects of different lactational estrus induction protocols on piglet performance are fairly well-characterized; yet data from larger populations may substantiate proposed benefits on the incidence of post-weaning diarrhea (IS) and the variation in pig body weight at marketing (SW).

Other limitations for lactational estrus implementation need also be considered. Seasonal infertility during summer and early autumn is likely to reduce responsiveness to lactational estrus induction attempts. Additionally, successfully mating primiparous sows during lactation at high rates currently appears unlikely. Farms utilizing group gestation systems must also consider the reproductive consequences of mixing time relative to mating date in lactationally-served sows. It is encouraging that the majority of induction protocols have reported normal wean-to-estrus intervals in sows failing to ovulate prior to weaning, potentially making management of these non-responders less complex.

This review hypothesized that weaning is no longer needed to start the next reproductive cycle. The physiological capabilities of contemporary sow lines combined with methods used in recent research imply that breeding in lactation can now achieve comparable reproductive rates to sows mated post-weaning. Nonetheless, numerous obstacles limiting widespread commercial implementation remain. Further research in several areas can improve upon present knowledge to develop practical, efficacious induction protocols.

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Chapter 2 - Suckling Reduction and Boar Exposure to Induce Estrus and Ovulation in Primi- and Multiparous Lactating Sows and Consequences for Litter Growth in the Peri-Weaning Period

ABSTRACT

Multiparous (MP) and primiparous (PP) sows (n=53) were exposed to boars and litter separation to determine the effects on sow reproduction and the growth and survival of their pigs through the nursery period. Litter size was equalized to 12.6 ± 1.2 pigs at d 2 post-farrowing and at d 18, sows were allotted to control or an altered suckling method (ALT). On d 18, the ALT sows were placed in adjacent pairs within parity and all but the 5 lightest BW pigs were split-weaned (SW) and moved to the nursery. The 10 lightweight pigs for each pair of sows formed a combined litter and rotationally-suckled (RS) each sow 12 h/d from d 18 to 25. Thus, pigs had nursing access 24 h/d but each ALT sow was only suckled 12 h/d. Daily boar exposure was also provided to ALT sows. Control sows continued to nurse their litters without modifications. Control and ALT litters were weaned at d 21 and d 25, respectively. Lactation BW and backfat losses were similar between treatments, although ALT sows had 16% greater total feed intake ($P < 0.01$) during lactation due to the extended lactation period. Primiparous sows lost a greater percentage (7.4 vs. 3.4%) of BW and consumed less ($P < 0.01$) feed than MP sows. A total of 25 of 28 ALT sows were detected in estrus and mated in lactation. Although the interval from initiating ALT to estrus was greater ($P < 0.001$) than the wean-to-estrus interval (WEI) for controls, ALT sows were in estrus earlier (23.0 vs. 24.6 d; $P < 0.001$) post-farrowing. Pregnancy rate and subsequent reproductive performance were similar. Pigs were weighed on d 18, 21, 25, 28, and 32 of age. Differences in BW gain, variation in growth, and the association between pig

BW category on d 18 and treatment effects were evaluated. An interaction was detected ($P <$ 0.01) for pig BW and weight gain from d 18 to 32 as the RS pigs gained 15% more than lightweight controls, whereas SW pigs were 15% lighter than heavyweight controls on d 32 leading to 50% less ($P < 0.01$) variation as measured by CV in ALT litters compared to controls. When pig BW groups were compared, the ALT treatment benefited ($P < 0.001$) growth of light (<4.5 kg) pigs but decreased ($P < 0.01$) BW gain of heavy (>6.4 kg) pigs. Overall, ALT sows expressed a high rate of lactational estrus with fertility similar to control sows and ALT litters responded with similar average growth but less variation than controls. The reduced BW variation for ALT litters warrants additional investigation.

INTRODUCTION

Sows experience a period of lactational anestrus driven by suckling-induced suppression of gonadotropin secretion (Quesnel and Prunier, 1995; Kemp et al., 2009). A minimum of 14 to 21 d is needed for uterine involution and resumption of reproductive activity (Polge, 1972; Varley, 1982); consequently, weaning currently occurs at least 2 wk postpartum and has moved closer to 3 wk to support better piglet performance and welfare (von Borell, 2000; Smith and Stalder, 2008).

Producers have significant incentives to shorten the interval from farrowing to conception (King et al., 1998). One way to circumvent the negative impact of early weaning is to uncouple weaning and rebreeding by mating during lactation, which may reduce sow non-productive days and increase lactation length.

Several strategies have been evaluated to elicit a fertile estrus in lactation. Earlier efforts yielded inconsistent responses, but showed that reduced nursing and boar exposure are important stimuli (Stevenson and Davis, 1984a; Newton et al., 1987; Costa and Varley, 1995). Recent

international research has re-visited these ideas to address welfare and production issues and results indicate that some current sow lines are more responsive than previously thought (Langendijk et al., 2009; Downing et al., 2012; Terry et al., 2014).

The primary objective was to determine whether an altered suckling treatment (ALT) and boar exposure could induce lactational estrus. By split-weaning (SW) the heavier pigs and commingling the remaining lightweight pigs in 2 adjacent litters, ALT provided continuous access to nursing but restricted sows to 12 h/d of suckling. Since reducing the suckling stimulus seems to be critical in motivating lactational estrus expression, a secondary objective aimed to characterize the effects of ALT on piglet growth. This treatment provides additional nursing for lightweight pigs but requires weaning larger littermates earlier. The effects of ALT on both weight groups are evaluated.

MATERIALS AND METHODS

Animals and Housing

This study was conducted with the approval of the Kansas State University Institutional Animal Care and Use Committee. All experimental procedures were conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS from the months of October through December 2012. The farrowing, gestation, and nursery barns used were totally enclosed, environmentally controlled, and mechanically ventilated buildings. A total of 53 sows (PIC 1050; Hendersonville, TN) and their litters were used in 2 consecutive groups, with 35 d between groups. In anticipation that primiparous (PP) sows would be less likely to respond with lactational estrus, they were separated from multiparous (MP) sows in the experimental design. Parity ranged from 1 to 5 and averaged 2.6 ± 1.5. On d 110 of gestation, each group of pregnant sows was moved into a single farrowing room that contained 29 individual farrowing crates

(2.13 × 0.61 m for the sow and an additional 2.13 × 0.96 m for the litter) arranged in 2 parallel rows. Sows not farrowing by d 115 of gestation were injected IM with dinoprost tromethamine (Lutalyse®; 10 mg; Zoetis Animal Health, Florham Park, NJ) to induce parturition. Litter size at birth varied from 3 to 18 live pigs and was equalized within 2 d after farrowing by cross-fostering pigs within each parity group, resulting in an average litter size of 12.6 ± 1.2 pigs. Pigs were individually weighed, ear-notched, and given iron dextran (200 mg) and ceftiofur sodium (Naxcel®; 50 mg; Zoetis Animal Health, Florham Park, NJ) within 24 h after farrowing. Male pigs were castrated approximately 7 d after birth. The day on which most of the litters were born was considered d 0 of lactation for the group, and all treatment procedures were performed on the same calendar day for all litters in the farrowing group. Litters were born between 4 d before to 3 d after d 0. Sows were fed the same lactation diet (3,245 kcal/kg, 21.6% CP, and 0.97% SID Lys) that contained corn, soybean-meal, and 20% DDGS. Lactation feed was provided ad libitum beginning the day after farrowing by individual Gestal Solo (JYGA Technologies, St. Lambert, Quebec, Canada) electronic sow feeders. Ad libitum water access was provided to sow and litter via cup waterer access at floor level. Creep feed was not offered during lactation. Temperature in the farrowing house was maintained at a minimum of 20°C, and supplemental heat was provided to piglets with heat lamps.

Estrus-behavior was tested during boar exposure for ALT sows. At weaning, sows were moved into pens of 6 to 8 sows and checked daily for estrus with a boar. All sows were examined by transrectal ultrasound for ovarian structures beginning on d 17 and after weaning sows were temporarily moved into individual gestation stalls each day for ultrasound. After weaning, sows in estrus were moved to individual gestation stalls (2.13 × 0.61 m) and fed 2.0

kg/d of a common corn and soybean meal-based gestation diet (3,241 kcal/kg, 14.1% CP, and 0.56% Lys).

Treatments

On d 18 of lactation, sows were allotted to treatments within parity group and with sow BW, suckled litter size (average 11.6 ± 1.2 pigs) and day of farrowing equalized as nearly as possible. A total of 25 control sows (16 MP and 9 PP) and 28 ALT sows (20 MP and 8 PP) were assigned to treatments. Control litters had continuous access to the sow until weaning. The ALT sows were placed in adjacent pairs within parity group such that 2 litters could be combined and rotated between sows by temporarily lifting the pen divider between farrowing crates. On d 18, all but the 5 lightest-weight pigs from each ALT litter were SW and moved to the nursery. The remaining 10 lightweight pigs on paired ALT litters were combined to form a new litter of 10 pigs. These combined litters were rotationally-suckled (RS) between paired sows at 12 h intervals (0600 and 1800 h), such that pigs had access to a sow 24 h/d, but each ALT sow was only suckled for 12 h/d. This regimen was applied from d 18 until ALT sows were weaned on d 25. Control sows were managed according to standard farm practice and their litters were weaned on d 21. To reduce any photoperiod effects, artificial lights remained on for 24 h/d throughout lactation and post-weaning until ovulation was confirmed in all experimental animals.

Beginning on d 18, ALT sows were provided daily exposure to a boar by moving the sow to a pen adjacent to the farrowing room. Each sow received approximately 5 min of fence-line contact followed by 5 min of full physical contact and a final 5 min of fence-line boar contact. To maximize stimulation, 1 of 3 mature boars was used for full physical contact on each day with a second boar providing fence-line contact. Boars were rotated each day to minimize

individual boar effects. Additionally, sows were presented to the boar in a different order each day. Boar exposure was provided from d 18 until ovulation or at weaning on d 25. Sow BW and backfat thickness measurements (Lean Meter®; Renco Corp., Minneapolis, MN) were recorded at entry to the farrowing crate, post-farrowing, and on d 18, 21, and 25 post-farrowing. Daily lactation feed intake was also recorded.

Reproduction

Standing estrus was confirmed using a back-pressure test in the presence of a boar. Sows were artificially inseminated at first observed estrus and again 24 h later. Inseminations were performed using a disposable spirette and each insemination contained approximately 70 mL of extended semen (<5 d old) purchased from a commercial boar stud (Zoltenko Farms Inc., Courtland, KS).

Pregnancy diagnosis was performed by transabdominal ultrasound (Hitachi-Aloka USA, Wallingford, CT) at 28 to 35 d after insemination. Sows that were not pregnant or were 5th parity or greater were removed from the herd according to standard farm practice and no further data were collected. The remaining 40 sows (20 control and 20 ALT) were retained and farrowing rate, total born, number born live, stillbirths, mummies, and birth weights were recorded for all resulting litters.

Follicular Measurements

Ovaries of sows were scanned by transrectal ultrasound using an Aloka 500V ultrasound with a 5.0-MHz linear transducer (Hitachi-Aloka USA, Wallingford, CT). From d 17 to 21, ultrasound was performed daily for ALT sows and every other day for control sows. After d 21, all sows were scanned daily until ovulation. Ovulation was considered to have occurred at 12 h prior to the ultrasound exam when less than 4 intact preovulatory follicles (usually 8 to 12 mm)

were found. At each scan, the number of follicles per ovary and the average diameter of the 3 largest follicles on each ovary was recorded. A sow was considered to have cystic follicles when multiple large structures with anechoic interiors and between 1 and 3 cm remained present for at least 5 d after estrus onset (Castagna et al., 2004). Single large cysts were detected occasionally and these were noted but not included in the follicle count. Single cysts are commonly observed in sows and apparently do not impact fertility (Ryan and Raeside, 1991). Therefore, these latter sows were not considered cystic.

Hormone Analysis

Blood was collected from all sows on d 18, 21, and 25 and 2 additional samples were collected 8 to 12 and 18 to 21 d post-estrus. Progesterone (P4) concentrations were used to determine whether ovulation had occurred prior to d 18 post-farrowing and to confirm ovulation after visual estrus detection as well as the establishment of pregnancy. Ovulation was assumed to have taken place when P4 exceeded 4.0 ng/mL (van de Wiel et al., 1981; Armstrong et al., 1999). Jugular vein blood was collected using 38-mm × 20-gauge needles and 10 mL blood collection tubes without additive (Covidien Ltd., Mansfield, MA). After clotting for 6 h, the serum was separated by centrifugation (1,600 × g for 25 min at 4°C) and stored (–20°C) until analysis by RIA. Serum estradiol-17β (E2; MP Biomedical, Solon, OH) and P4 (Coat-A-Count, Siemens Medical, Los Angeles, CA) were analyzed in duplicate using commercial RIA kits. Assay sensitivity was 0.6 pg/mL for E2 and 0.01 ng/mL for P4. Intra- and inter-assay CV were 14.20 and 6.87%, respectively, for E2 and 1.00 and 2.38%, respectively, for P4. For both P4 and E2, adding increasing volumes of serum produced a curve that paralleled the standard curve and the parallelism and average mass recoveries were 109.8% and 103% for P4 and E2, respectively.

Piglet Measurements

Weaned pigs were allotted to pens within treatment by BW and gender with 7 pigs per pen. Nursery pens (1.2 × 1.5 m) had woven wire flooring, a 3-hole, dry self-feeder, and a nipple waterer to allow for ad libitum access to feed and water. Regardless of weaning age, piglets were fed according to the same feed budget consisting of 1.8 kg/pig of a commercial Phase 1 diet followed by a Phase 2 diet until the end of the experiment. Piglet BW was recorded at birth and at d 18, 21, 25, 28 and 32.

Data Analysis and Statistics

Data are presented as least squares means ± SEM. All normally distributed data were analyzed using a general linear mixed model (Version 9.4, SAS Institute, Inc., Cary, NC). Treatment means were compared using least significant differences. Fixed effect factors were treatment (Control; n = 25, ALT; n = 28) and parity (PP; n = 17, MP, n = 36) as well as their interactions. Sow was the experimental unit and farrowing group (n = 2) was included in the model as a random effect. For serum E2 analysis, the statistical model was the same except sample collection d, treatment × sample collection d and treatment × parity × sample collection d were additional fixed effects. Sample collection d was analyzed as a repeated measure with sow as the subject. Conception rate was evaluated by χ^2 analysis using the LOGISTIC procedure of SAS.

For piglet performance, pigs originating from both control and ALT sows were compared by separating the 5 lightest BW control pigs into a light BW category corresponding to the RS pigs from ALT litters, whereas the remaining heavyweight pigs in control litters were compared against the SW pigs from ALT litters. Pig was the experimental unit with nursery pen and farrowing group included as random effects. Within litter, CV for growth rate was compared between control and ALT litters using litter as the experimental unit. A post hoc analysis was

also applied to evaluate the association between d 18 piglet BW group and treatment. For this comparison, individual pigs were the experimental unit and pigs were retrospectively assigned to 1 of 4 BW classifications based on d 18 BW: <4.5 kg, 4.5 to 5.4 kg, 5.4 to 6.4 kg, or >6.4 kg. Total BW gain and the average BW of pigs within each weight group were then compared across treatments. Least squares mean differences were evaluated using pairwise comparisons between treatments within BW classification. Differences among treatments were considered significant at $P \leq 0.05$ and marginally significant if $P > 0.05$ and $P \leq 0.10$.

RESULTS

Sows

No treatment × parity interactions were observed for sow BW, feed intake, or BF through d 25 post farrowing. The ALT sows were heavier and had greater backfat ($P < 0.01$) at d 25 (Table 1), but when adjusted for weaning age, control and ALT sows had similar BW and backfat losses during lactation. Average daily feed intake was similar between treatments, but due to a longer lactation length, ALT sows had 16% greater ($P < 0.01$) total feed intake during lactation. No differences were detected in piglet mortality during the 7 d treatment period.

Primiparous sows had lighter ($P < 0.001$) BW than MP sows before farrowing and remained lighter throughout lactation. Primiparous sows also lost a greater ($P < 0.01$) percentage of BW during lactation and tended ($P < 0.10$) to lose more BW than MP sows. Both ADFI and total feed intake were less ($P < 0.001$) for PP than MP sows.

A total of 19 of 20 MP and 6 of 8 PP sows in the ALT treatment were detected in estrus and inseminated during lactation (Table 2). Evaluations of P4 concentrations in serum of sows at 8 to 12 and 18 to 21 d post-estrus revealed that 2 multiparous ALT sows failed to establish

pregnancy after ovulation, and a third multiparous ALT sow failed to remain pregnant to pregnancy determination by ultrasound. Among ALT sows not detected in estrus during lactation, the remaining MP sow was in estrus on the day of weaning (d 25), and the remaining 2 primiparous ALT sows were detected in estrus and mated at 9 and 12 d after the initiation of the ALT treatment (2 and 5 d after weaning). Based on P4 concentrations >4.0 ng/mL, 1 MP control sow ovulated prior to treatment initiation at d 18 and thus was not detected in estrus. Despite having ovulated, this sow, along with 14 of 16 other MP and all 9 PP sows, was detected in estrus and mated post-weaning. The remaining MP control sow had more than 4 follicles with diameters greater than 15 mm without ovulating for 3 d and appears to have had cystic ovarian follicles.

No treatment × parity interactions were detected for sow reproductive performance (Table 3). The wean-to-estrus interval (WEI) was shorter (3.8 vs 5.4; $P < 0.001$) for controls than the time from initiation of ALT to estrus. However, when expressed as the days from farrowing to estrus, ALT sows were detected in estrus quicker (23.4 vs. 24.8 d; $P < 0.001$) than controls. For both treatments, PP sows were in estrus later (5.4 vs. 3.8 d; $P < 0.01$) than MP sows. Figure 1 shows the distribution of estrus and Figure 2 illustrates the cumulative percentage of sows in estrus over time for both treatments. No treatment or parity differences were detected for conception rate.

The subsequent litters produced by control and ALT sows did not differ statistically (Table 4). There was a tendency for a treatment × parity interaction ($P < 0.10$) for the percentage of mummified fetuses, but the limited number of sows and variation in this trait make interpretation difficult. Pigs farrowed by parity 2 sows (initially PP) tended ($P < 0.10$) to be heavier than pigs from MP sows.

For serum E2 concentrations, there were no 4 or 3-way interactions among treatment, parity, day and farrowing group. A treatment × day interaction was present (quadratic, $P < 0.001$) where E2 increased from d 18 to 25 in control sows, but increased rapidly and then decreased in ALT sows. While no treatment × parity group interactions were present for E2, PP sows had lower ($P < 0.01$) E2 concentrations than MP sows at d 21.

Ultrasound observations of follicular development generally corresponded with observed E2 and estrus observations between treatments and parity groups (Table 5). A treatment × parity interaction ($P < 0.05$) occurred because MP control sows reached maximum follicle diameter and ovulated quicker ($P < 0.05$) post-weaning than PP controls and ALT sows irrespective of parity. However, when expressed as days post-farrowing, ALT sows reached maximum follicle diameter more rapidly ($P < 0.05$) than controls. The diameter of the largest follicles increased after ALT and weaning and by d 21 was greater for ALT sows (Figure 3). As illustrated in Figure 4, PP sows responded with slower growth in follicle diameter. Accordingly, PP sows ovulated later ($P < 0.05$) than MP sows.

Pig Performance

Pigs nursing control and ALT sows were similar in BW at allotment on d 18. However, an interaction was detected ($P < 0.01$) for each subsequent time point and for weight gain from d 18 to 32 in which RS pigs gained more weight than lightweight control pigs but SW pigs were lighter compared with the initially heavyweight controls (Figure 5). Comparing the collective performance of ALT pigs versus controls showed that although control pigs were heavier than ALT pigs at d 21.5, weights were similar at each subsequent time point, and the total gain from d 18 to 32 did not differ between the two suckling treatments. The RS pigs were lighter ($P < 0.001$)

than their SW counterparts at each time point, and the lightweight pigs within the control group remained lighter ($P < 0.001$) than the heavyweight control pigs.

Figure 6 depicts the change in CV within each litter from d 18 to 32. Litters where the ALT suckling treatment was applied had decreased ($P < 0.05$) variation at d 21.5 and d 32 corresponding to a greater reduction ($P < 0.01$) of CV relative to control litters over the 14 d period.

As shown in Figure 7, of the piglets that were <4.5 kg on d 18, those subjected to ALT were heavier ($P < 0.01$) than controls on d 25 and 32 and experienced greater ($P < 0.001$) BW gain from d 18 to 32. Conversely, pigs >6.4 kg and subjected to ALT were lighter ($P < 0.01$) at d 21.5, d 28.5, and d 32 and experienced less ($P < 0.01$) BW gain compared with controls. The 4.5 to 5.4 kg controls were heavier ($P < 0.05$) on d 21.5 than their ALT counterparts, but otherwise pigs within the 4.5 to 5.4 kg and 5.4 to 6.4 kg categories performed similarly regardless of the suckling treatment applied.

DISCUSSION

Sows typically remain anestrus throughout lactation. Piglet proximity and teat stimulation cause the release of endogenous opioid peptides (EOP) in the brain and EOP suppress secretion of luteinizing hormone (LH) by inhibiting the gonadotropin-releasing hormone (GnRH) pulse generator (De Rensis et al., 1993; De Rensis et al., 1999). This EOP-induced LH suppression, combined with the negative energy balance typical during lactation (Quesnel et al., 1998; Van den Brand et al., 2001), normally prevents follicles from reaching ovulatory size during lactations of 21 to 28 d. However, LH pulsatility is gradually restored as lactation progresses, which may be attributed to decreased suckling frequency combined with an increase in pituitary responsiveness to GnRH and increases in releasable LH pools within the pituitary (Sesti and

Britt, 1993; Quesnel, 2009; Soede and Kemp, 2015). Accordingly, in contemporary hyperprolific sow lines, a small percentage of sows are able to escape the suckling-induced LH suppression and ovulate during lactation (Langendijk et al., 2009; Downing et al., 2012; Terry et al., 2014). This phenomenon appears to be more likely to occur in MP sows suckling small litters, especially when the sow has high lactation feed intake and an extended weaning age (>21 d; Kemp and Soede et al., 2012). This pattern is consistent with observations of the present study, where P4 analysis revealed that 1 MP control sow, nursing only 7 piglets, ovulated prior to d 18.

Earlier research demonstrated that a reduction of the suckling stimulus is necessary to elicit lactational estrus, but inconsistent sow responses prevented industry-wide adoption (Smith, 1961; Stevenson and Davis, 1984a; Newton et al., 1987). Methods used to reduce the suckling stimulus include SW, where a portion of the litter (usually the heaviest pigs) are weaned several days prior to the remaining piglets, as well as intermittent suckling (IS), where all piglets are temporarily separated from the sow for a period of time each day. When combined with daily boar exposure, recent work with SW (Terry et al., 2013; Terry et al., 2014) or IS (Gerritsen et al., 2009; Downing et al., 2012) has yielded lactational ovulation rates as high as 90 to 100% without detriment to subsequent reproductive performance. However, prior to this experiment, lactational estrus induction in sows in the United States had not been revisited since the 1980s. Therefore, the major aim of this study was to evaluate the receptivity of US sows to lactational estrus using a novel suckling reduction method (ALT) combining elements of SW and IS. Previous research by Britt and Levis (1982) indicates that the WEI is decreased when paired sows alternately nursed 2 entire litters for 48 h prior to weaning. We hypothesized that ALT, which combines alternate suckling and SW would further reduce the suckling effect while creating an opportunity for lightweight pigs to benefit from additional nursing access. The rotation of lightweight pigs

between paired sows required approximately 1 to 2 min/litter and was performed by 1 worker at 0600 and 1800 h by lifting the divider between adjacent litters and encouraging pigs into the adjacent crate. No piglet injuries or deaths were observed resulting from interactions with non-parent sows.

Early reports also revealed that boar exposure alone is sufficient to stimulate estrus in some lactating sows (Rowlinson and Bryant, 1975; Stolba et al., 1990), and in recent studies, boar contact alone elicited estrus in more than half (55 to 67%) of lactating sows (Terry et al., 2013; van Wettere et al., 2013; Terry et al., 2014; Weaver et al., 2014). However, since reproductive rates suffered when boar exposure alone was provided, the best responses have been observed when daily boar contact is accompanied by other components of a lactational estrus induction regimen (e.g. suckling reduction, exogenous hormones, grouping lactating sows). The use of a mature boar is also critical for successful estrus detection (Hemsworth et al., 1990; Langendijk et al., 2000). Thus, in the present study, 15 min of combined full and fence-line boar contact was incorporated into the ALT treatment. Only 1 farrowing room was available so ALT sows were removed from farrowing crates each day and walked approximately 30 m to an outdoor pen for boar contact. This limited potential effects of boar contact on control sows.

Overall, the results of the present study show that ALT can stimulate a high rate of lactational estrus and ovulation (89%), similar to control sows with litters weaned completely. The rate of lactational estrus we observed is greater than many reports in the literature and this may be related to the sow line used, time of year, and unique aspects of the ALT treatment. In addition to reduced hours of nursing each day, the ALT sows were nursed by a combined litter of foreign and own pigs that were lightweight compared with the litter nursing before treatment. These foreign pigs may be perceived in a way that contributes to the occurrence of estrus, but

further work will be required to evaluate individual components of the treatment. It is of note that of the 3 ALT sows failing to show estrus in lactation, 1 MP sow was found in estrus the day of weaning and the 2 PP sows were in estrus 2 and 5 d post-weaning. While few 'non-responders' were available for comparison, these observations are congruent with earlier claims by Stevenson and Davis (1984b) that sows respond to IS regimens in an 'all or none' fashion, with non-responders typically showing a normal WEI (Soede et al., 2015).

Aside from the single sow that ovulated prior to d 18, no controls ovulated prior to weaning on d 21, although the WEI was shorter (3.8 ± 1.4 d) than typical for this farm (5 to 7 d). The proximity of controls to adjacent ALT sows in estrus and residual boar pheromones on those sows may have contributed to a more rapid estrus onset. In weaned sows, provision of a female in estrus adjacent to anestrus sows is known to reduce the WEI (Pearce and Pearce, 1992). As shown in Figure 1, the 25 of 28 sows responding to ALT treatment did so in a synchronous fashion, with most sows in estrus 4 to 6 d after the beginning of ALT. While ALT sows did not respond as rapidly as the WEI for controls, ALT sows were still found in estrus 1.4 d sooner post-farrowing, with no detriment to conception rate.

The occurrence of lactational estrus was greater in MP sows compared to PP (95 vs. 75%), which is consistent with previous reports (Stevenson and Davis, 1984a; Newton et al., 1987a; Newton et al., 1987b; Soede et al., 2012). This reduced response is likely due in part to the lower lactation feed intake and greater BW loss typical of PP sows, known to negatively affect reproductive performance even when sows are conventionally-weaned (Koketsu et al., 1996; Thaker and Bilkei, 2005; Hoving et al., 2011). In accordance with previous reports, PP sows lost more BW during lactation, had less ADFI during lactation, and onset of estrus occurred later, but this effect was present regardless of treatment. However, the 4 d longer lactation

resulted in greater overall feed disappearance for ALT sows who consumed approximately 23 kg more lactation feed than controls.

Recent IS studies (reviewed by Soede et al., 2015) indicate reduced pregnancy rate and embryo survival as well as impaired embryo development can occur if lactational ovulation occurs as early as 19 to 21 d post-partum or if IS continues for 20 d beyond ovulation, possibly related to reduced P4 concentrations in these sows (Gerritsen et al., 2008a). Moreover, Langendijk et al. (2007a) and Mattioli et al. (1988) reported differential LH release patterns depending on whether the sow was housed out of sight and sound from the piglets during separation. While the present study was designed to limit additional labor requirements by utilizing the adjacent sow's crate as the separation area, it was otherwise designed to adhere to recommendations by Gerritsen et al. (2008b). According to Gerritsen et al. (2008b), subsequent reproductive performance should be similar to sows mated conventionally post-weaning if lactational mating occurs beyond 21 d after farrowing and IS does not extend beyond 9 d post-mating. Recent experiments complying with those guidelines have reported fertility levels similar to conventional mating practices (Gaustad-Aas et al., 2004; Downing et al., 2012; Soede et al., 2012). In this study, the ALT sows mated in lactation had similar conception rate compared to control sows, but due to the farm's culling practices, any 5th parity sows (n = 7) or non-pregnant sows (n = 6) were removed from the herd after pregnancy determination. For the remaining 40 sows, subsequent reproductive rates were similar regardless of treatment. While the numbers of sows remaining were likely insufficient to make definitive conclusions, the data collected provide indication that the ALT treatment did not significantly alter subsequent litter characteristics.

Transrectal ultrasound was performed on all sows to evaluate patterns of follicular growth in ALT sows and to confirm the time of ovulation. While single large ovarian cysts (1 to 3 cm) were recorded in 7 controls and 5 ALT sows, these are known to cause little interference with cycle length or litter size (Ryan et al., 1991). Multiple large ovarian cysts (1 to 3 cm), characteristic of infertility and abnormal estrous behavior, were only observed in 1 MP control sow who showed estrous behavior 7 d post-weaning but failed to ovulate. The absence of cystic ALT sows indicates that beginning ALT on d 18 is an adequate post-farrowing interval for fertility. Incomplete uterine involution (Palmer et al., 1965; Varley and Cole, 1978) and inadequate pituitary LH stores (Bevers et al., 1981; Sesti and Britt, 1993) have been considered the main limiting factors of initiating lactational estrus earlier post-partum. When an IS regimen was implemented at d 14 of lactation, sows were more likely to develop cystic follicles (Gerritsen et al., 2014). However, Downing et al. (2011) reported similar reproductive rates regardless of initiation day (d 14, 16, or 18 post-partum) when IS was combined with gonadotropin injection and boar exposure.

As shown in Figure 3, the ALT sows ovulating during lactation displayed a pattern of follicular growth similar to but accelerated compared to controls. Serum E2 concentrations at d 18, 21, and 25 substantiate these follicular patterns, as ALT sows reached peak E2 at d 21, whereas E2 levels in controls continued to increase to d 25. The similar maximum follicle diameter and follicle diameter at ovulation between ALT and control sows is consistent with sows subjected to a 12 h IS regimen (Gerritsen et al., 2008). Delayed follicular development and onset of estrus after weaning is typical of PP sows, and seasonal infertility can exacerbate this effect (Britt et al., 1985). Since this experiment was conducted in October to December, the effects of season were likely minimal. While decreased responses to lactational estrus induction

are typical for PP sows (Stevenson and Davis, 1984a; Terry et al., 2014), to our knowledge, this is the first report where follicular growth differences have been reported between PP and MP sows subjected to a lactational estrus induction regimen. As expected, PP controls had delayed follicular development compared to MP controls, but interestingly, for the ALT sows ovulating during lactation there were no differences in follicular development due to parity. This may be influenced by the low number of primiparous ALT sows (n = 6) included in the comparison, but may indicate that the PP sows that are capable of ovulating during lactation have follicular growth rates similar to MP sows. This is consistent with the 'all or none' phenomena proposed by Stevenson and Davis (1984b). The 2 PP sows failing to ovulate during lactation both displayed follicle growth from 3.5 to 6 mm prior to weaning, but these follicles failed to develop to preovulatory size (7 to 8 mm) prior to weaning. After weaning, a new cohort of follicles appeared which then ovulated normally. Conventionally-weaned sows with extended WEI often display this same pattern of follicular growth, known to be more prevalent in first parity sows (Bracken et al., 1999; Langendijk et al., 2000; Lucy et al., 2001). Additional work may confirm these observations on larger numbers of animals.

The ALT treatment was also designed to potentially offset disadvantages and capitalize on advantages observed with other suckling reduction strategies such as IS and SW. While IS reduces the severity of post-weaning growth suppression compared to abruptly weaned pigs, pigs subjected to IS for 12 h/d are typically lighter BW at weaning and similar in BW at the end of the nursery period (Kuller et al., 2004; Berkeveld et al., 2007; Kuller et al., 2007). Moreover, IS requires additional labor, especially if the pigs are removed from sight and sound of the sows, as is recommended for optimal sow response (Langendijk et al., 2007b). Split weaning is more easily integrated into the current weaning practices of a herd (Matte et al., 1992) and improves

the BW gain of lightweight pigs, although the growth benefit is also generally transitory (Mahan, 1993; Pluske et al., 1996). While some recent SW experiments have yielded rates of lactational estrus comparable to those achieved using IS (75 to 93%; Terry et al., 2013; Terry et al., 2014), others have been less promising (48%; Kirkwood et al., 2013) and SW has been speculated as contributing to reduced subsequent litter size (Terry et al., 2014). Using adjacent sows in the ALT treatment to RS lightweight pigs provides lactating sows a temporal suckling reduction akin to IS, but execution of ALT by lifting the divider between crates is less laborious compared to gathering pigs daily and removing them to a separate area as in IS.

A unique component of the ALT treatment is the co-mingling of lightweight piglets from 2 litters prior to weaning. Previous studies have reported benefits to co-mingling prior to weaning including reduced aggression (Weary et al., 2002; Parratt et al., 2006), faster establishment of a dominance hierarchy (D'Eath, 2005), and increased post-weaning weight gain (Weary et al., 2002). The additional 3.5 d of nursing access prior to weaning and the co-mingling prior to weaning may have contributed to RS pigs being heavier BW at d 32 and having 15% greater total weight gain relative to lightweight controls. However, the benefits to RS pigs were offset by reduced growth in SW pigs, as they experienced a more marked post-weaning growth check, resulting in 15% poorer total gain compared with heavyweight controls. This reduced growth rate may be explained in part by the earlier weaning age (Main et al., 2004), but also may be related to the fact that SW pigs were grouped together at weaning whereas heavyweight control pigs were housed alongside lightweight controls. Combining the lighter SW pigs and heavier RS pigs showed a 50% reduction in BW variation at d 32 for ALT versus control litters. Additional research is needed to determine whether the improvement in variation is maintained through the finishing period, but there is some indication of long-term growth benefit, as Mahan

(1993) reported lightweight SW pigs reached market 2 days quicker than lightweight pigs conventionally weaned.

Further evaluation of d 18 weight categories revealed that overall differences between treatments occurred primarily because of changes in the BW of pigs in the <4.5 and >6.4 kg categories. As seen in Figure 7, <4.5 kg ALT pigs experienced 15% more gain than controls, but for the >6.4 kg group, ALT pigs were 8% lighter. It is logical that the lightest pigs may benefit most from the additional time on the sow with reduced competition from their heavier littermates. However, it is intriguing that of the heavier-weight groups, pigs >6.4 kg experienced the biggest setback in performance by weaning at d 18 rather than d 21.5. When creep feeding is practiced, heavyweight pigs within a litter at weaning may be slower to consume dry feed post-weaning because they, unlike lightweight pigs, typically have unrestricted access to nursing opportunities prior to weaning (Pajor et al., 1991; Sulabo et al., 2010).

Overall, the current findings demonstrate that ALT is a promising strategy to induce estrus and ovulation in lactating sows with fertility rates similar to sows mated conventionally post-weaning. The ALT sows were detected in estrus more quickly after farrowing than the controls. Previous lactational estrus work with primiparous sows is limited, and the present data suggests that estrus in lactation also can be stimulated in these sows; moreover, the altered suckling method did not negatively affect litter performance in the peri-weaning period.

Future research may help develop practical protocols that allow breeding during lactation, but additional work is necessary to confirm these results in larger populations of sows and to determine the most effective and practical presentation of stimuli. Treatments similar to this study may benefit lightweight pigs in large litters, and breeding during lactation could help

enhance group sow housing management. Because individual farrowing stalls are more accepted for the welfare advantages to the nursing pigs, this last benefit is worth exploring further.

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Table 2.1. Interactive effects of an altered suckling treatment (ALT) with boar exposure on lactational characteristics of multi- and primiparous sows[1]

	Multiparous		Primiparous		Main Effects		Probability, $P <$[1]	
Item	Control	ALT	Control	ALT	Control	ALT	Trt	Parity
Sows, n	16	20	9	8	25	28	---	---
Parity	3.3	3.4	1.0	1.0	2.4	2.7	---	---
Piglets suckling, d 18	11.8±0.3	11.7±0.3	11.3±0.4	11.3±0.4	11.5±0.3	11.4±0.2	0.780	0.260
d 18 litter weight, kg	66.9±2.7	66.6±2.4	62.4±3.6	61.2±3.8	65.4±2.2	65.2±2.2	0.983	0.290
Sow BW, kg								
d 1 post-farrowing	253.8±11.9	254.6±11.5	210.7±13.3	206.0±13.7	238.3±11.3	240.7±11.3	0.863	0.001
d 18 post-farrowing	244.0±7.9	247.8±7.5	200.7±9.6	196.2±10.0	228.4±7.2	233.1±7.2	0.881	0.001
d 21 post-farrowing	242.6±9.1	246.2±8.7	197.6±10.6	190.0±10.9	226.4±8.5	230.1±8.5	0.974	0.001
d 25 post-farrowing	219.4±8.6	243.3±8.1	180.8±10.0	183.9±10.8	205.5±7.9	227.9±8.1	0.013	0.001
Lactation BW change, kg[3]	-11.2±3.3	-8.5±3.1	-13.4±3.9	-17.9±4.3	-12.0±3.0	-13.2±3.1	0.852	0.059
Lactation BW change, %[3]	-4.3±1.2	-3.1±1.1	-6.1±1.4	-8.7±1.6	-4.9±1.1	-4.7±1.1	0.978	0.008
Sow backfat, mm								
d 1 post-farrowing	13.3±1.0	14.2±0.9	15.4±1.2	14.8±1.2	14.1±0.9	14.4±0.9	0.615	0.126
d 18 post-farrowing	12.7±0.7	13.3±0.6	13.8±1.0	12.6±1.0	13.1±0.6	13.1±0.6	0.988	0.796
d 21 post-farrowing	12.6±0.8	12.6±0.7	14.4±1.0	12.8±1.1	13.2±0.7	12.6±0.7	0.533	0.266
d 25 post-farrowing	11.3±0.7	13.8±0.6	12.3±0.9	12.6±1.0	11.6±0.6	13.5±0.6	0.015	0.965
Lactation backfat change, mm[3]	-2.0±1.2	-0.4±1.2	-3.1±1.3	-2.2±1.4	2.5±1.2	-0.9±1.2	0.113	0.556
Lactation ADFI, kg[4]	5.8±0.3	5.8±0.2	4.8±0.3	4.2±0.4	5.4±0.2	5.3±0.2	0.715	0.001
Lactation feed intake, kg[4]	122.6±7.0	147.0±6.5	102.4±8.6	110.1±9.0	115.5±6.2	136.5±6.2	0.004	0.001

[1] A total of 53 sows (PIC 1050) were used across two farrowing replicates. Sows were allotted to treatments on d 18 of lactation. Controls were weaned on d 21; whereas ALT sows were split-weaned to the 5 lightest BW pigs on d 18. The remaining 5 pigs were combined between 2 adjacent sows and these 10 pigs were rotated between sows at 12 h intervals.

[2] No treatment × parity group interactions were detected ($P > 0.108$).

[3] Lactation BW and backfat change measured from d 1 post-farrowing to 21 for controls and d 1 to 25 for ALT sows.

[4] Incorporates feed intake from actual farrowing date for each sow.

Table 2.2. The number of multiparous and primiparous sows exhibiting lactational estrus and ovulation in control and ALT sows

	Multiparous		Primiparous	
Item	Control	ALT	Control	ALT
Sows, n	16	20	9	8
Sows mated during lactation	0	19	0	6
Ovulated during lactation[1]	1[2]	19	0	6
Pregnant at d 18 to 21 post-estrus[3]	1	17	0	6
Pregnant at d 28 to 35 post-estrus[4]	0	16	0	6
Cystic ovaries	0	0	0	0
Sows mated post-weaning	16	1	9	2
Ovulated[1]	15	1	9	2
Pregnant at d 18 to 21 post-estrus[3]	15	1	8	2
Pregnant at d 28 to 35 post-estrus[4]	15	1	8	1
Cystic ovaries	1	0	0	0

[1] Serum progesterone > 4.0 ng/mL on d 8 to 12 d post-estrus.
[2] This sow had elevated progesterone on d 18 post-farrowing. This sow also expressed post-weaning estrus and she appears twice in the table.
[3] Serum P4 > 4.0 ng/mL.
[4] Determined at 28 to 35 d post-estrus using transabdominal ultrasound.

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Table 2.3. The effects of altered suckling (ALT) with boar exposure on the reproductive performance of multi- and primiparous lactating sows

	Multiparous		Primiparous		Main Effects		Probability, $P <$[1]	
Item	Control	ALT	Control	ALT	Control	ALT	Trt	Parity
Sows, n	16	20	9	8	27	28		
Weaning or initiation of ALT to estrus, d	3.1±0.4	4.5±0.3	4.4±0.5	6.4±0.5	3.8±0.3	5.4±0.4	<0.001	<0.001
Day in estrus after farrowing	24.1±0.4	22.5±0.3	25.4±0.5	24.4±0.5	24.8±0.3	24.0±0.4	<0.001	<0.01
Conception rate,[2] %	93.8	90.0	88.9	86.0	92.0	89.0	0.71	0.69

[1] No treatment × parity group interactions were detected ($P > 0.543$).

[2] Based on transabdominal ultrasound at 28 to 35 d after insemination. χ^2 analysis was conducted using PROC LOGISTIC in SAS (SAS Institute, Inc., Cary, NC) to compare treatment means.

Table 2.4. The effects of altered suckling (ALT) with boar exposure on subsequent reproductive performance of multi- and primiparous sows

	Multiparous		Primiparous		Main Effects		Probability, $P <$	
Item	Control	ALT	Control	ALT	Control	ALT	Trt	Parity
Sows retained, n[2]	13	14	7	6	20	20		
Total born	13.1±1.2	12.8±1.2	12.1±1.5	11.5±1.6	12.6±1.1	12.1±1.1	0.66	0.32
Number born live	12.8±1.2	12.2±1.1	11.7±1.5	11.5±1.5	12.2±1.0	11.8±1.1	0.63	0.42
Stillbirths, %	6.1±2.7	7.8±2.6	5.3±3.6	3.4±3.9	5.7±2.2	5.6±2.3	0.87	0.44
Mummies, %	2.3±1.3	4.3±1.3	4.0±1.8	0.0±0.0	3.1±1.1	2.1±1.2	0.97	0.50
Piglet BW, kg	1.37±0.07	1.47±0.07	1.57±0.10	1.58±0.10	1.47±0.06	1.53±0.06	0.40	0.08
Litter weight, kg	16.9±1.42	17.7±1.40	17.8±1.76	17.4±1.86	17.4±1.31	17.6±1.33	0.74	0.85

[1]All non-pregnant or parity 5 or greater sows were culled and removed from the experiment.

[2] No treatment × parity group interactions were detected ($P > 0.543$).

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Table 2.5. The interactive effects altered suckling (ALT) with boar exposure or weaning on follicle development and ovulation for sows ovulating within 7 d after weaning or ALT[1]

	Multiparous		Primiparous		Main Effects		Probability, $P <$		
Item	Control	ALT	Control	ALT	Control	ALT	Trt × Parity	Trt	Parity
Sows, n	14	20	9	6	23	26			
Estradiol-17β,[3,4] pg/mL									
Day 18	6.8±1.0	6.3±0.9	4.5±1.1	6.1±1.2	5.9±0.9	6.3±0.9	0.147	0.437	0.098
Day 21	20.1±5.0	40.9±4.4	9.1±6.0	22.5±7.1	15.8±4.3	36.6±4.5	0.480	0.002	0.007
Day 25	17.0±4.7	8.4±3.9	22.8±5.8	8.4±7.9	19.3±3.8	8.4±4.4	0.621	0.054	0.621
Follicle development[5]									
Initial follicle diameter, mm	3.9±0.4	3.9±0.4	3.8±0.4	3.5±0.5	3.9±0.4	3.7±0.4	0.351	0.415	0.158
Maximum follicle diameter, mm	8.3±0.3	8.4±0.2	8.4±0.3	8.1±0.4	8.3±0.2	8.3±0.3	0.593	0.796	0.679
Follicle diameter at ovulation, mm	8.0±0.3	8.2±0.2	8.0±0.4	7.9±0.5	8.0±0.2	8.1±0.3	0.597	0.983	0.690
Day of max. follicle diameter after ALT or wean	2.9±0.3	5.0±0.3	4.7±0.4	4.8±0.5	3.6±0.3	5.0±0.3	0.017	0.007	0.055
Day of max. follicle diameter after farrowing	23.9±0.3	23.0±0.3	25.7±0.4	22.8±0.5	24.8±0.3	22.9±0.3	0.017	0.001	0.055
Time to ovulation after ALT or wean, h[6]	93±7	136±6	137±9	137±12	110±6	136±7	0.017	0.020	0.012

[1] Removed from analysis: 2 ALT primiparous sows that failed to ovulate within 7 d, 1 control sow that ovulated prior to d 18, and 1 control sow with cystic ovaries.

[2] No treatment × parity × day interactions ($P < 0.723$) were detected. A treatment × day interaction was detected (quadratic, $P < 0.001$) where estradiol-17β increased from d 18 to 25 in control sows, but increased rapidly to d 21, then decreased in ALT sows.

[3] There was an increase (quadratic, $P < 0.001$) in estradiol-17β from d 18 to 25.

[4] Daily transrectal ultrasound (500V, 5.0 MHz; Aloka, Wallingford, CT) measurements were collected from d 17 until 7 d postweaning. Follicle diameter reported as the average of the 3 largest follicles on each ovary.

[6] Time of ovulation was defined as 12 h prior to the ultrasound exam when fewer than 4 preovulatory follicles remained between both ovaries.

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Table 2.6. The effects of altered suckling (ALT) with boar exposure on piglet BW during late lactation and the early nursery period

	Control[2]			ALT[2]				Probability $P <$		
Item	Heavy[3]	Light[3]	Total	SW	RS	Total	SEM	Trt × BW category	Trt	BW category
Pigs, n	164	125	289	183	139	322				
Weaning age, d	21.5	21.5		18	25					
Pig BW, kg										
d 18	6.25	4.80	5.53	6.29	4.85	5.57	0.078	0.977	0.620	0.001
d 21.5	7.14	5.52	6.33	6.55	5.71	6.13	0.073	0.001	0.031	0.001
d 25	7.74	6.10	6.92	7.62	6.54	7.08	0.098	0.006	0.119	0.001
d 28.5	8.68	6.91	7.79	8.33	7.16	7.75	0.115	0.007	0.677	0.001
d 32	9.76	7.84	8.80	9.46	8.27	8.87	0.267	0.003	0.595	0.001
Gain d 18 to 32, kg	3.51	3.03	3.27	3.17	3.43	3.30	0.330	0.001	0.677	0.075

[1] A total of 611 pigs (PIC 327 × 1050) originating from 53 litters in 2 farrowing replicates were used in this 14-d study with 7 pigs per pen after weaning. Birth weights of pigs averaged 1.41 ± 0.3 kg and were similar between control and ALT treatments.

[2] Sows were allotted to 1 of 2 treatments at d 18 of lactation based on parity, sow weight, suckled litter size, and average piglet weight. The altered suckling treatment (ALT) involved split-weaning (SW) all but the 5 lightest BW pigs on d 18. The ALT sows were then paired and the lightweight pigs from 2 litters were combined and rotationally suckled (RS) between the pair of sows at 12 h intervals until weaning on d 25.

[3] Pigs from control sows were weaned on d 21.5 (afternoon of d 21) and allotted to nursery pens by BW and gender. Although litters remained intact until weaning, control pigs are sorted into “Heavy” and “Light” categories using d 18 BW and the criteria applied to ALT litters.

8

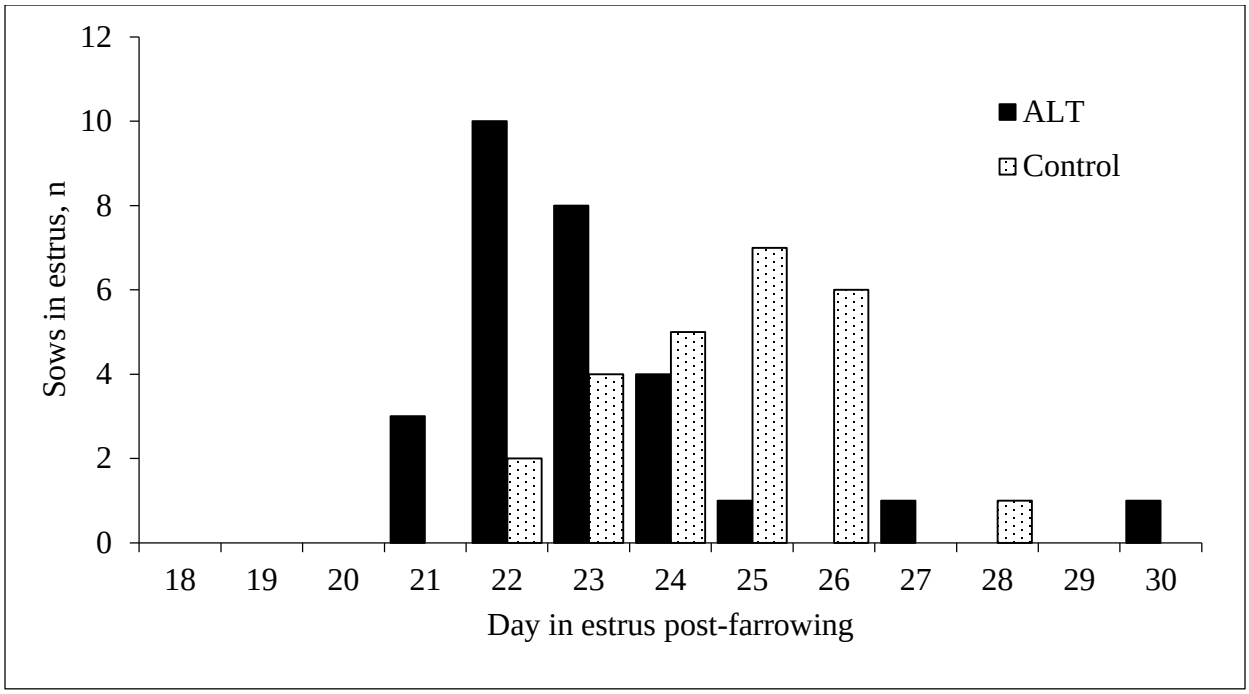

Figure 2.1. The day of first detected estrus for control sows and sows provided boar exposure and an altered suckling treatment (ALT).

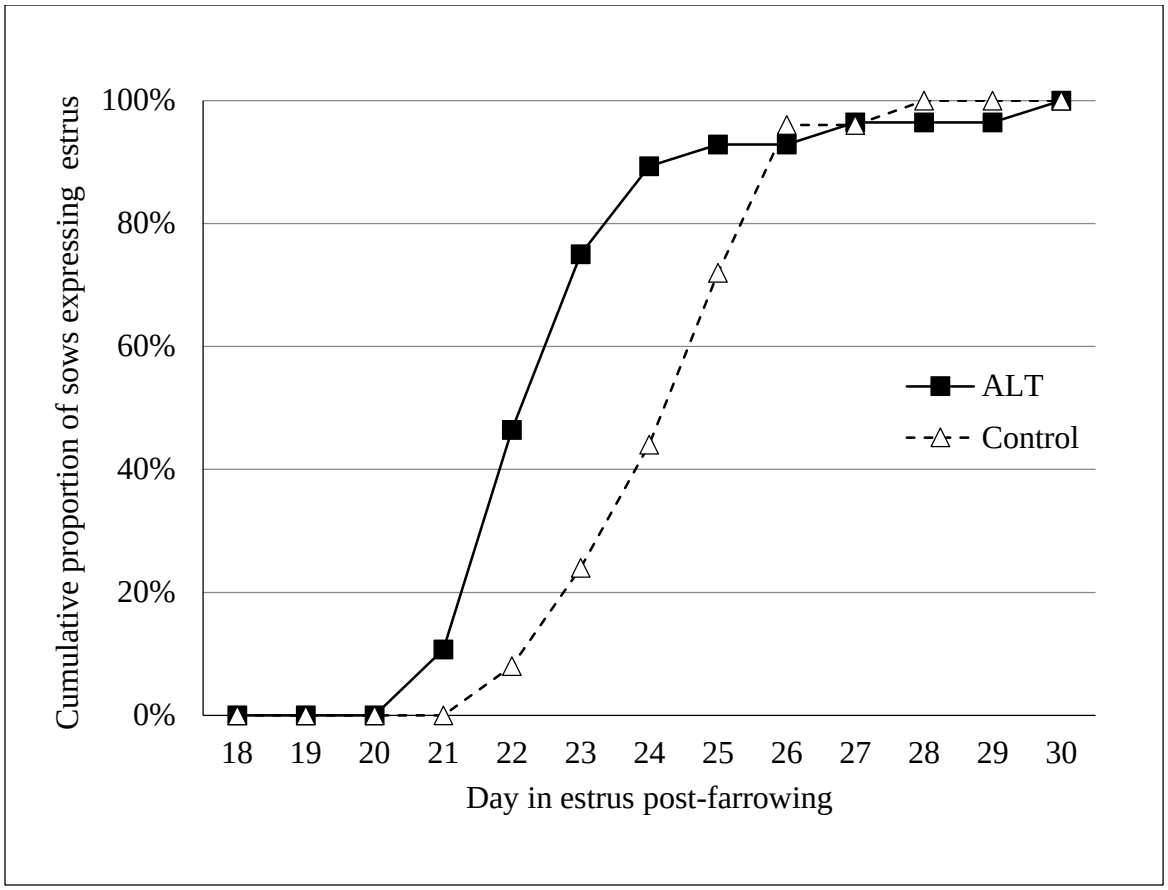

Figure 2.2. The cumulative percentages of control and ALT sows in estrus post-farrowing.

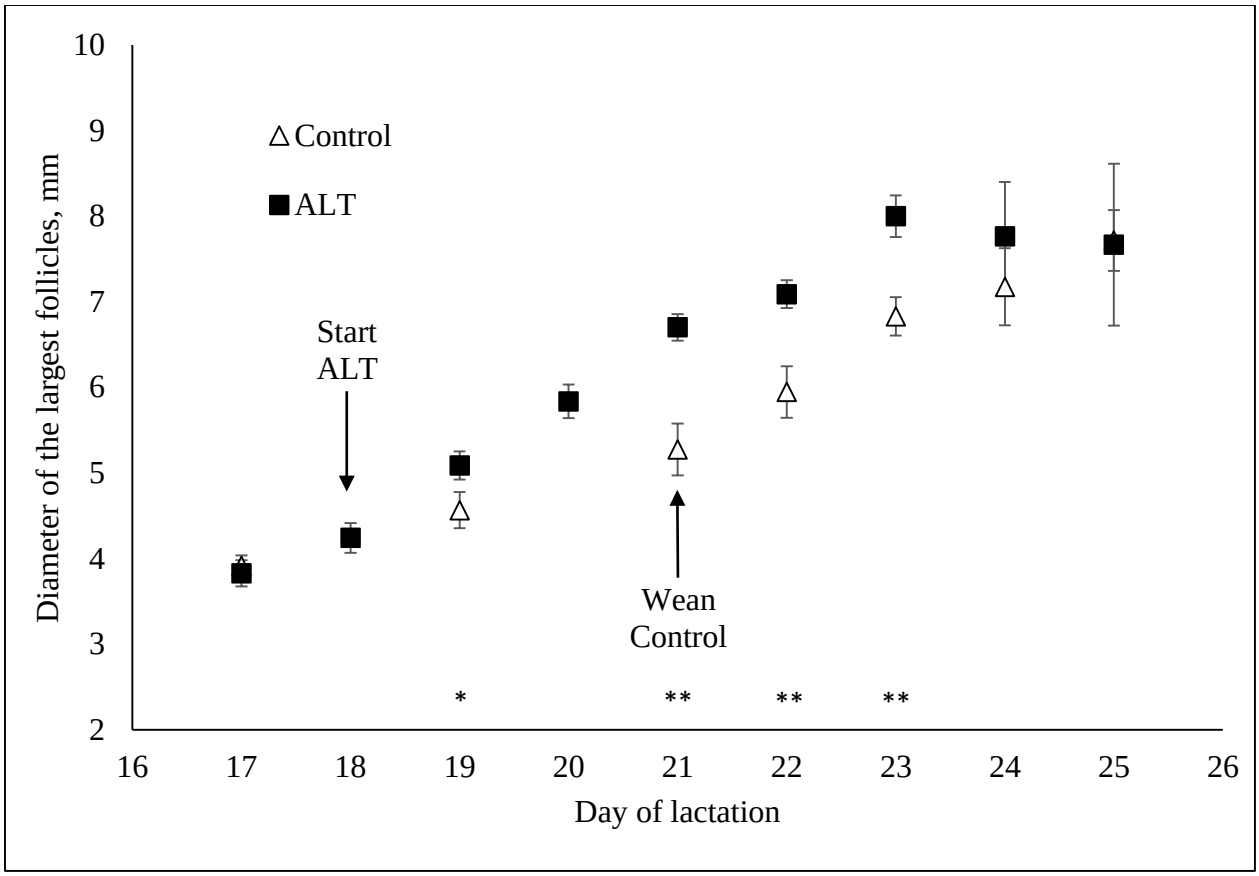

Figure 2.3. Change in the follicle diameter of the largest follicles (mean and standard errors) in response to altered suckling (ALT) with boar exposure or weaning. * $P < 0.10$. ** $P < 0.05$.

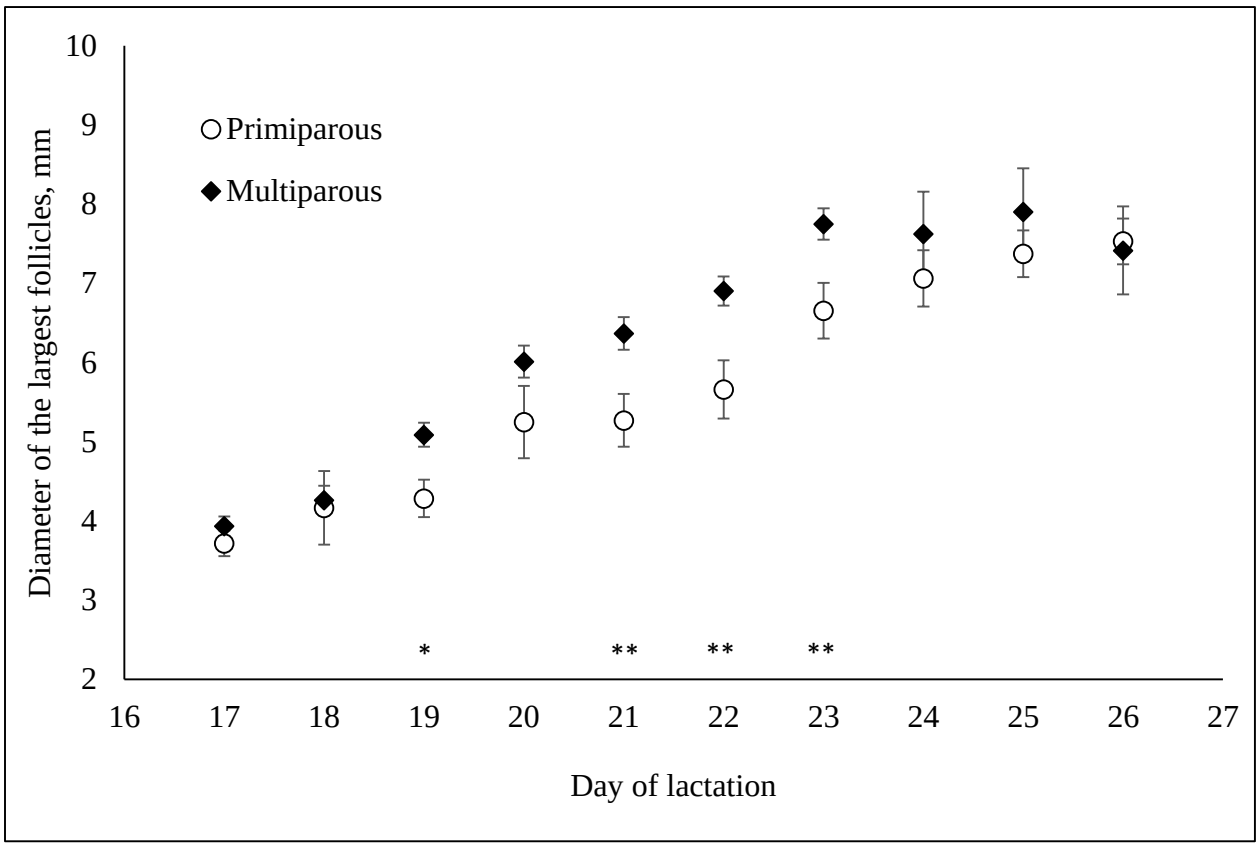

Figure 2.4. Change in mean follicle diameter of the largest follicles after treatment (d 18 of lactation) for multiparous and primiparous sows. * $P < 0.10$. ** $P < 0.05$.

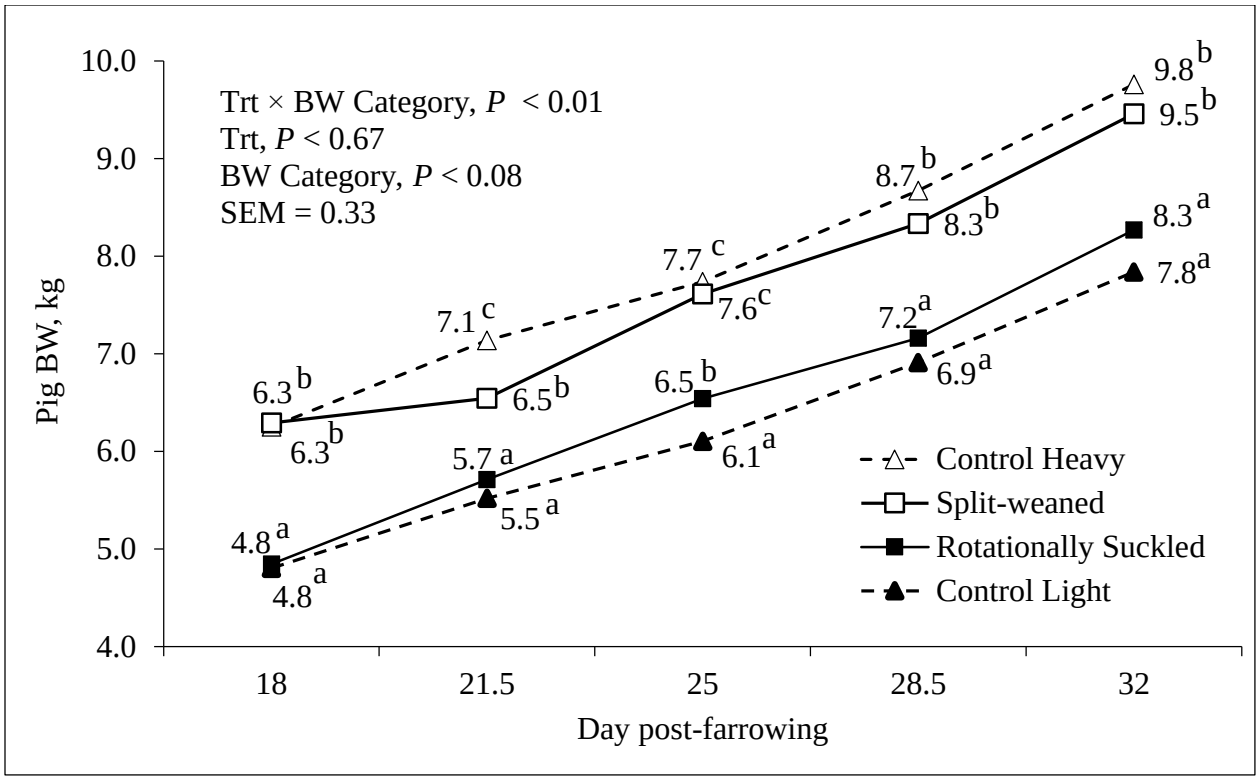

Figure 2.5. The effects of altered suckling (ALT) with boar exposure on piglet BW during late lactation and the early nursery period. [a,b,c] Means without a common superscript differ P < 0.05.

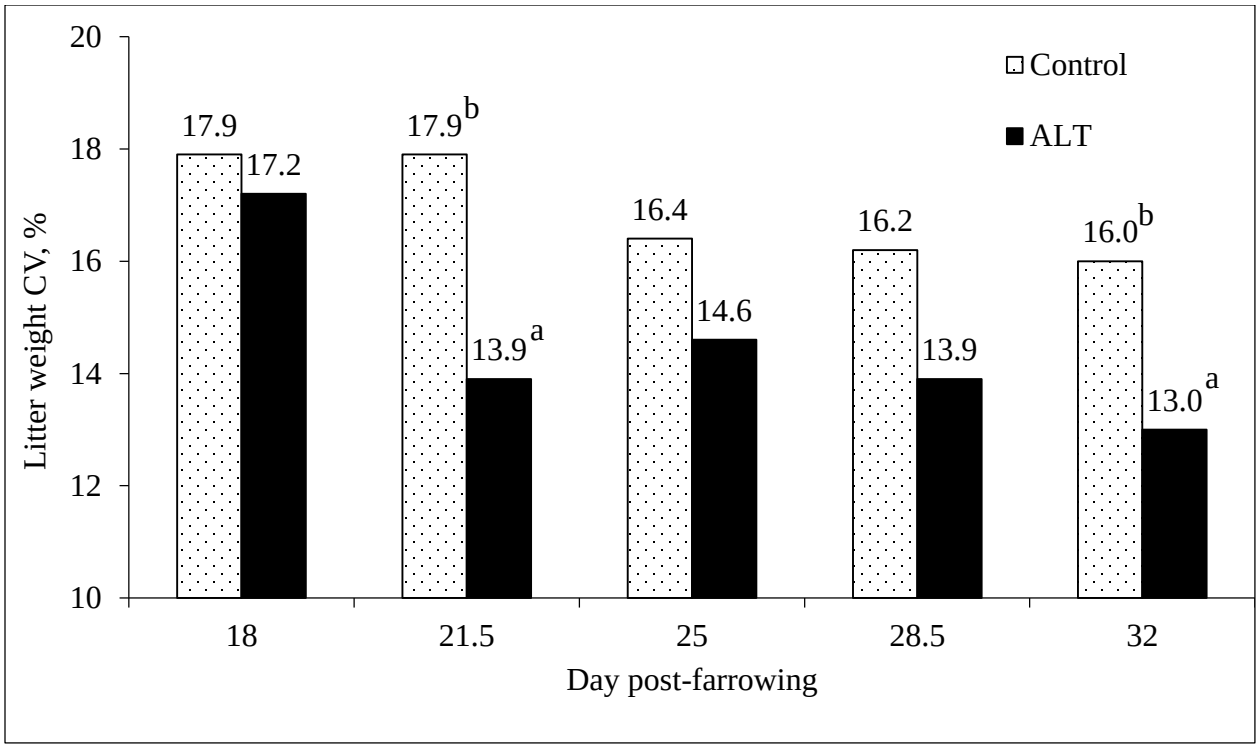

Figure 2.6. The effects of an altered suckling treatment (ALT) on piglet BW variation within litter during late lactation and the early nursery period. A total of 25 control and 28 ALT litters were included with an average litter size at d 18 of 11.56 and 11.60 pigs.

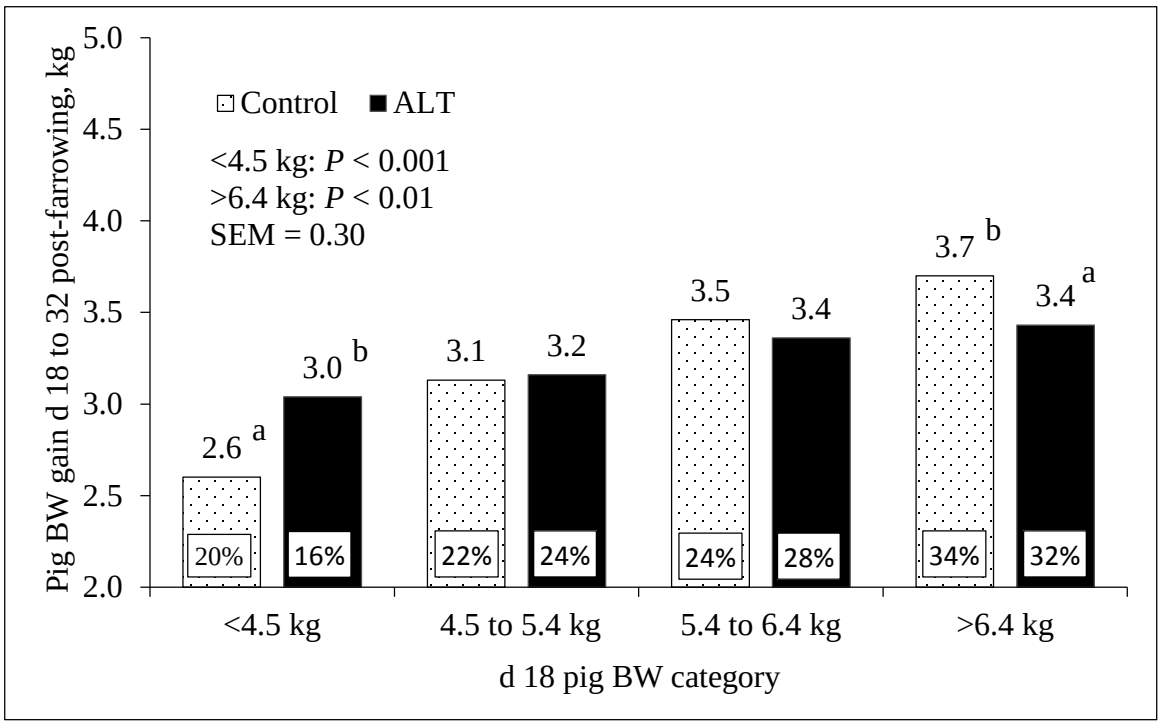

Figure 2.7. The effects of an altered suckling treatment (ALT) on pig BW gain from d 18 to 32 for different d 18 BW categories. Numbers within data bars indicate the percentage of piglets falling within each BW category for control and ALT. [a,b] Means without a common superscript differ, $P < 0.05$.

Chapter 3 - A Comparison of Suckling Reduction Strategies to Enhance Estrus Induction in Boar-Exposed Lactating Sows and Effect on Performance Responses of Offspring to Market

ABSTRACT

A total of 135 sows (PIC 1050), ranging from parity 1 to 5 (2.6 ± 1.4), were used in 5 consecutive farrowing groups (Feb to Aug). The objective of the study was to evaluate different suckling reduction strategies on the incidence of lactational estrus and the effects on sow fertility and piglet growth. Litter size was equalized within parity (11.5 ± 1.1 piglets) at d 2 after farrowing. At d 18, sows were assigned to 1 of 5 treatments (n = 26 to 28) based on parity, farrowing date, and suckled litter size. Treatments were: 1) Control; 2) ALT (sows placed in adjacent pairs, on d 18 all but the 5 lightest piglets were weaned and remaining piglets combined and alternated between sows at 12 h intervals from d 18 to 25; 3) SEP (piglets separated for 12 h/d from d 18 to 25); 4) Split-wean (SW; all but the 5 lightest piglets weaned on d 18); and 5) 24HR (piglets separated from sows for 24 h on d 18). Controls were weaned at d 21 and all other treatments weaned at d 25. All sows were provided nose-to-nose contact with a mature boar for 5 min/d from d 18 until weaning without removing them from farrowing crates. Creep feed and water access was provided from d 14 to weaning. Offspring ADG was recorded to market for two farrowing groups. Sow backfat and BW losses during lactation were similar across treatments. Of 106 sows subjected to suckling treatments, 80 (76%) expressed lactational estrus. The SEP and 24HR sows were in estrus earlier ($P < 0.05$) than SW sows. A tendency for reduced conception rate in SEP and 24HR sows was observed ($P < 0.10$) versus control and SW sows.

Creep feed disappearance was greatest ($P < 0.01$) for SEP and 24HR litters and pig ADG from d 18 to 32 was reduced ($P < 0.05$) for these treatments. While unexpected differences in carcass yield and percentage lean were found, we failed to detect any negative effects of the reduced suckling treatments on final BW. In conclusion, altered suckling treatments differ in their ability to induce lactational estrus and impact on offspring gain immediately post-weaning, but did not influence offspring growth to market weight.

INTRODUCTION

Traditionally, weaning is the start of the reproductive cycle in sows. However, breeding sows during lactation is an alternative approach which may increase annual sow productivity (Kemp and Soede, 2012a). If sows conceive while lactating, farrowing interval and herd non-productive days may decrease, thereby increasing the number of litters/sow/yr (Kirkwood and Thacker, 1998). Early attempts to breed sows during lactation yielded inconsistent results (Crighton, 1970; Stevenson and Davis, 1984a; Newton et al., 1987a), and the longer wean-to-estrus interval (WEI) in sows at the time may have contributed to the limited success (Aumaitre et al., 1976; Britt and Levis, 1982). However, these studies showed that a consistent feature of successful lactational ovulation is the reduction of the suckling stimulus, alleviating endogenous opioid peptide-mediated suppression of LH and resulting in follicular development. Methods used include temporary daily separation of the litter, referred to as intermittent suckling (IS), or permanent removal of a portion of the litter via split-weaning (SW).

Recent attempts have been more successful (Gerritsen et al., 2009; Terry et al., 2013; Terry et al., 2014). Optimal responses have been observed when suckling reduction is combined with daily boar exposure. Applied properly, these induction strategies can elicit lactational ovulation in excess of 90% of sows with no detriment to subsequent reproductive performance or

litter growth. An altered suckling method (ALT), combining elements of IS and SW, also yielded positive results in a recent study (Frobose et al., 2013), and may aid in reducing variation in pig BW. However, limited data exists directly comparing these suckling reduction methods, and questions remain around the most practical method to apply on farms.

Thus, the objective was to compare suckling reduction strategies in boar-exposed lactating sows to induce lactational estrus and to assess litter growth to market weight.

MATERIALS AND METHODS

Animals and Housing

This study was conducted with the approval of the Kansas State University Institutional Animal Care and Use Committee. All experimental procedures were conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS from the months of February through August 2014. The farrowing, gestation, and nursery barns used were totally enclosed, environmentally controlled, and mechanically ventilated buildings. A total of 135 sows (PIC 1050; Hendersonville, TN) and their litters were used in 5 consecutive farrowing groups. Parity ranged from 1 to 5 and averaged 2.6 ± 1.4. On d 110 of gestation, pregnant sows were moved into a single farrowing room which contained 29 individual farrowing crates (2.13 × 0.61 m for the sow and an additional 2.13 × 0.96 m for the litter) arranged in 2 parallel rows. Sows not farrowing by d 115 of gestation were injected IM with dinoprost tromethamine (Lutalyse®; 10 mg; Zoetis Animal Health, Florham Park, NJ) to induce parturition. Litter size at birth varied from 6 to 18 live pigs and was equalized within 2 d after farrowing by cross-fostering pigs, resulting in an average litter size of 11.5 ± 1.1 pigs. Pigs were individually weighed, ear-notched, and given intramuscular injections of 2 mL iron dextran and 1 mL of ceftiofur sodium (Naxcel®; Zoetis Animal Health, Florham Park, NJ) within 24 h post-farrowing. Male pigs were castrated

approximately 7 d after birth. The day on which most of the litters were born was considered d 0 of lactation for the group, and all treatment procedures were performed on the same calendar day for all litters in the farrowing group. Litters were born between 4 d before to 2 d after d 0. Sows were fed a common lactation diet (3,233 kcal/kg, 19.8% CP, and 1.11% total Lys) which was corn-soybean meal based and fed in meal form. Lactation feed was provided ad libitum beginning the day after farrowing by individual Gestal Solo (JYGA Technologies, St-Nicolas, Quebec, Canada) electronic sow feeders. Ad libitum water access was provided to sow and litter via cup waterer access at floor level. Temperature in the farrowing house was maintained at a minimum of 20°C, and supplemental heat was provided to piglets with heat lamps. To reduce any photoperiod effects, artificial lights remained on for 24 h/d throughout lactation and post-weaning until ovulation was confirmed in all experimental animals.

From d 14 until weaning, a common commercial nursery diet was offered in a rotary creep feeder (Rotechna Mini Hopper Pan, Rotechna SA, Agramount, Spain). The creep diet was fed in pellet form (2-mm pellets), and sufficient amounts of creep feed were maintained in the 6-L hopper to ensure that feed was always available. The creep feeder was place in the middle of the side of the farrowing crate such that continuous creep access would be available for litters temporarily separated from sows due to experimental design. To provide a supplemental water source for piglets during litter separation events, a 1-L gravity-fed nipple waterer was mounted at pig height in the separation area between 2 sows and refilled twice daily from d 14 until weaning.

Estrus-behavior was tested during daily boar exposure for ALT sows. At weaning, sows were moved into pens of 6 to 8 sows and checked daily for estrus with a mature boar. Sows from 2 farrowing groups (n = 53, 9 to 12 sows per treatment) were examined daily by transrectal

ultrasound for ovarian structures beginning on d 17. After weaning, sows were temporarily moved into individual gestation stalls each day for ultrasound. Sows in estrus post-weaning were then moved to individual gestation stalls (2.13 × 0.61 m) and fed 2.0 kg/d of a common corn and soybean meal-based gestation diet (3,241 kcal/kg, 14.1% CP, and 0.56% Lys).

Treatments

On d 18 of lactation for each farrowing group, sows were allotted to 1 of 5 treatments (n = 26 to 28) with parity, d 18 litter size (average 11.3 ± 1.2 pigs) and day of farrowing equalized as nearly as possible. Treatments were: 1) Control; 2) Altered suckling (ALT); 3) Litter separation (SEP); 4) Split-weaning (SW); and 5) 24 h litter separation (24HR). Control sows were managed according to standard farm practice and were continuously suckled by the litter until weaning on d 21. For sows in the 4 reduced suckling treatments, treatment commenced on d 18 and continued until weaning on d 25. The ALT sows were placed in adjacent pairs within the farrowing room such that 2 litters could be combined and rotated between sows by temporarily lifting the pen divider. On d 18, all but the 5 lightest-weight pigs from each ALT litter were SW and moved to the nursery. The remaining 10 lightweight pigs on paired ALT litters were combined to form a new litter of 10 pigs. These combined litters were rotationally-suckled (RS) between paired sows at 12 h intervals (0600 and 1800 h), such that pigs had access to a sow 24 h/d, but each ALT sow was only suckled for 12 h/d. Sows in the SEP treatment were also placed in adjacent pairs so that 2 complete litters could be combined during the daily 12 h (0600 to 1800 h) separation period in a common area (2.13 × 0.96 m) created by removing the original crate divider and attaching new dividers to the sides of each sow's individual crate. From 1800 to 600 h, dividers were removed and all pigs could move freely between the paired sows. For SW sows, all but the 5 lightest-weight pigs were weaned and moved to the nursery. The remaining pigs

were allowed continuous nursing access until weaning. Sows in the 24HR treatment were paired as in ALT and SEP treatments so that the pigs from the 2 adjacent 24HR litters could be combined in the common area between 2 crates as in the SEP treatment. On d 18, 24HR piglets were separated from the sow for a single 24 h period, after which 24HR pigs were placed back with their original sow and allowed to continuously nurse until weaning.

Beginning on d 18, all sows were provided daily nose-to-nose contact to a boar by moving a mature boar into the center aisle of the farrowing room between the 2 rows of center-facing farrowing crates. The boar was harnessed to a remote controlled boar cart (BoarBot; Swine Robotics Inc., Leola, SD) and the boar positioned between farrowing crates to deliver sows the most sensory access to the boar. Each sow received approximately 5 min of contact. To minimize individual boar effects, 2 mature boars were rotated daily. Boar exposure was provided in this fashion from d 18 until ovulation or weaning. Sow BW and backfat thickness measurements were recorded at entry to the farrowing crate, post-farrowing, and on d 18, 21, and 25 post-farrowing. Daily lactation feed intake was also recorded.

Reproduction

Standing estrus was confirmed using a back-pressure test in the presence of a boar. Sows were artificially inseminated at first observed estrus and again 24 h later. Lactational and post-weaning inseminations were performed in the crate using post-cervical artificial insemination delivered during the refractory period immediately following standing estrus. Each insemination contained approximately 70 mL of extended semen (<5 d old) purchased from a commercial boar stud (Zoltenko Farms Inc., Courtland, KS). Progesterone concentrations were used to determine whether ovulation had occurred prior to d 18 post-farrowing and to confirm ovulation after visual

estrus detection. Ovulation was assumed to have taken place when P4 exceeded 4.0 ng/mL (van de Wiel et al., 1981; Armstrong et al., 1999)

Pregnancy diagnosis was performed by transabdominal ultrasound (Hitachi-Aloka USA, Wallingford, CT) at 28 to 35 d after insemination. After pregnancy determination, sows were either culled or allocated to another experiment and due to confounding treatment effects, subsequent reproductive performance could not be collected. Progesterone concentration was used to confirm establishment of pregnancy.

Follicular Measurements

For all sows in 2 farrowing groups (replicates 2 and 4), transrectal ultrasound was performed once daily using an Aloka 500V ultrasound with a 5.0-MHz linear transducer (Hitachi-Aloka USA, Wallingford, CT) from d 17 until ovulation. Ovulation was considered to have occurred at 12 h prior to the ultrasound exam when less than 4 intact preovulatory follicles (usually 8 to 12 mm) were found. At each scan, the number of follicles per ovary and the average diameter of the 3 largest follicles on each ovary was recorded. A sow was considered to have cystic follicles when multiple large structures with anechoic interiors and between 1 and 3 cm remained present for at least 5 d after estrus onset (Castagna et al., 2004). Single large cysts were detected occasionally and these were noted and but not included in the follicle count. Single cysts are commonly observed in sows and apparently do not impact fertility (Ryan and Raeside, 1991). Therefore, these latter sows were not considered cystic.

Hormone Analysis

Blood was collected from all sows on d 17, 21, and 25 and 2 additional samples were collected 8 to 12 and 18 to 21 d post-estrus to verify ovulation and confirm pregnancy recognition by extended elevated progesterone (P4), respectively. Jugular vein blood was

collected using 38-mm × 20-gauge needles and 10 mL blood collection tubes without additive (Covidien Ltd., Mansfield, MA). After clotting for 6 h, the serum was separated by centrifugation (1,600 × g for 25 min at 4°C) and stored (–20°C) until analysis by RIA. Serum estradiol-17β (E2; MP Biomedical, Solon, OH) and P4 (Coat-A-Count, Siemens Medical, Los Angeles, CA) were analyzed in duplicate using the commercial RIA kits validated in Chapter 1. Assay sensitivity was 0.6 pg/mL for E2 and 0.06 ng/mL for P4. Intra-assay CV was 6.45 and 5.92%, for E2 and P4, respectively.

Piglet Measurements

Pig growth performance to market was measured for all litters from 2 of the 5 farrowing groups (54 litters, 626 pigs). Weaned pigs were allotted to pens within treatment by BW and gender with 7 pigs per pen. Nursery pens (1.2 × 1.5 m) had woven wire flooring, a 3-hole, dry self-feeder, and a nipple waterer to allow for ad libitum access to feed and water. Regardless of weaning age and sow treatment, pigs were fed according to the same feed budget consisting of 1.4 kg/pig of a commercial Phase 1 pelleted diet followed by 5.4 kg/pig of Phase 2 diet and then Phase 3 until the end of the nursery phase (d 49). After exiting the nursery phase, pigs were moved to an on-site grower facility for 21 d prior to beginning the finishing phase. In both the grower and finisher facilities, pigs from each treatment were distributed as evenly as possible. Pig BW was recorded at birth and at d 18, 21, 25, 28, 32, 49, and 170. On d 170, pigs were weighed immediately prior to transport (approximately 204 km) to a commercial abattoir (Triumph Foods Inc., St. Joseph, MO). Pigs were individually tattooed according to pen number to allow for data retrieval by pen and carcass data collection at the abattoir. Standard carcass criteria of percentage carcass yield, HCW, back fat depth, loin depth and percentage lean were measured. Percentage lean was calculated according to NPPC (1991) equations for lean-

containing 5% fat, where lean (5% fat) = {2.83 + [0.469 x (0.4536 x HCW)] - [18.47 x (0.0394 x fat depth)] + [9.824 x (0.0394 x loin depth)] / (0.4536 x HCW)}. Hot carcass weights were measured immediately after evisceration, and percentage yield was calculated by dividing HCW by live BW obtained at the farm prior to transport.

Data Analysis and Statistics

Data in tables and figures are presented as least squares means ± SEM. Normally distributed data were analyzed using a general linear mixed model (Version 9.4, SAS, SAS Institute, Inc., Cary, NC). Sow was the experimental unit. The model included the fixed effect of treatment and random effects of farrowing group by treatment and farrowing group as a random effect. Conception rate and lactational estrus rate were evaluated by χ^2 analysis using the GLIMMIX procedure of SAS; however, controls were excluded from the lactational estrus analysis due to lack of variance because no control sows exhibited estrus during lactation. For serum E2 analysis, the statistical model was the same except day of bleeding and treatment × day of bleeding served as fixed effects in addition to treatment. Day of bleeding also served as the repeated measure with sow as the subject. Further analysis was done by categorizing follicular growth on the suckling reduction treatments (n = 53). Sows were classified as: "responders" if follicle growth > 6 mm was observed with ovulation in < 7 d after initiating suckling reduction, "non-responders" if follicle growth > 6 mm did not occur within 7 d, and as "abnormal" if follicle growth > 6 mm progressed within 7 d, but the sow failed to ovulate.

When comparing pig growth, BW and BW variation within litter (CV) among the reduced suckling treatments, d 18 pig BW was used as a covariate. The control, SEP, and 24HR litters remained intact until weaning and individual pigs categorized as heavy and light using d 18 BW and included in the statistical model. Similar to the pigs chosen for the ALT and SW

litters, the 5 lightest were categorized as light and the remainder were categorized as heavy. For evaluations of growth after d 18, clustering within litter was accounted for by using litter within farrowing group and the effect of nursery pen as a random effect. For carcass performance the fat depth, loin depth, and lean percentage were adjusted to a common HCW using HCW as a covariate.

Differences among means were compared using pairwise comparisons. Individual mean comparisons were protected with an overall treatment probability of $P < 0.10$. Then individual treatment differences among treatments were considered significant at $P \leq 0.05$ and marginally significant if $P > 0.05$ and $P \leq 0.10$.

RESULTS

No treatment × farrowing group interactions were observed for sow BW, feed intake, or backfat loss through d 25 post farrowing (Table 1). Sows in the 4 reduced suckling treatments were heavier ($P < 0.05$) than controls at d 25 post-farrowing, and ALT sows had greater ($P < 0.05$) backfat depth at d 25 versus control and SEP sows, with SW and 24HR sows intermediate. The SEP, SW and 24HR sows consumed more ($P < 0.05$) total lactation feed than controls; however, when adjusted for different lactation lengths, lactation ADFI was similar across treatments and no differences were observed for backfat or BW change during lactation. No differences in piglet mortality were detected during the 7 d application of suckling reduction treatments.

Despite receiving boar exposure from d 18 to 21, no control sows were in estrus or ovulated during lactation (Table 2). Based on P4 concentrations >4.0 ng/mL, 24 of 25 controls ovulated within 7 d post-weaning. According to P4 analysis, 1 ALT sow ovulated prior to d 18 post-farrowing and she was removed from the dataset. A total of 21 of 27 ALT sows were in

estrus during lactation, with 18 of those sows ovulating based on P4. Of the 3 ALT sows with lactational estrus but failing to ovulate, 2 continued to show estrus behavior for 6 and 7 d consecutively, and the third sow returned to estrus 4 d after weaning. Of 26 SEP sows, 19 exhibited estrus behavior during lactation and P4 concentrations indicate that 15 of those sows ovulated. Two of the 4 anovulatory SEP sows based on P4 were detected in estrus within 7 d after weaning. According to P4 analysis, all 23 of the SW sows exhibiting lactational estrus also ovulated. The 24HR treatment yielded 17 of 27 sows with lactational estrus, but 4 of these sows failed to ovulate during lactation and exhibited estrus behavior again 3 to 4 d after the initial estrus was observed. All 27 of the sows not responding with lactational estrus in the 4 reduced suckling treatments showed estrus within 7 d of weaning, and 26 of those sows ovulated based on serum P4 levels.

No treatment × farrowing group interactions were present with regard to onset of estrus and conception rates (Table 3). Three sows were removed from the analysis due to death, ovulation prior to allotment, and an ulcer. For the remaining sows, the incidence of lactational estrus was not significantly different between reduced suckling treatments, ranging from 63 to 85%, with similar conception rates between those sows lactationally mated. Of sows in estrus during lactation, SEP and 24HR sows responded with lactational estrus more rapidly ($P < 0.05$) after d 18 compared to SW sows, with ALT sows intermediate. For sows exhibiting estrus post-weaning, the WEI was similar regardless of treatment. However, the overall conception rate for 24HR sows was lower ($P < 0.05$) than controls or SW sows, with ALT and SEP sows intermediate.

Sows within farrowing group 2 and 4 (9 to 12 sows/trt) were ultrasounded daily from d 17 to ovulation (Table 4). Among those sows, all 10 control sows developed preovulatory size

follicles (> 6 mm) within 7 d of weaning, but collectively, 6 of the 41 sows in reduced suckling treatments did not respond to suckling reduction with follicular growth > 6 mm in the first 7 d. Over half of sows (26 of 41) in the 4 reduced suckling treatments, responded to treatment initiation on d 18 with follicular growth in excess of 6 mm and ovulation within 7 d. While no control or SW sows showed "abnormal" follicle growth, 2 ALT sows, 5 SEP sows, and four 24HR sows, developed preovulatory follicles within 7 d of treatment initiation, but failed to ovulate. The 2 ALT sows remained in estrus for an extended period (6 to 7 d) and then ovulated. Three SEP and two 24HR sows fitting this categorization developed follicles > 6 mm, but these follicles regressed without ovulating. A further 2 sows in both SEP and 24HR treatments appeared to ovulate based on ultrasound, but then showed estrus within 7 d post-weaning and ovulated after the post-weaning estrus.

For ultrasounded sows ovulating within 7 d of suckling reduction or weaning (Table 5), a treatment × day interaction was detected (quadratic, $P < 0.001$) for E2 responses, where estradiol-17β increased from d 18 to 25 in control sows, but increased to d 21, then decreased (quadratic, $P < 0.001$) in sows ovulating in response to reduced suckling treatments. This coincides with the more rapid ($P < 0.05$) follicle growth to > 6 mm for sows in the 4 reduced suckling treatments. While other follicular characteristics were similar regardless of treatment, control sows ovulated more quickly ($P < 0.05$) after developing preovulatory-sized follicles than sows in reduced suckling treatments. Moreover, onset of estrus and ovulation occurred more rapidly ($P < 0.05$) relative to weaning in control sows than the rate of estrus onset in sows responding to initiation of suckling reduction.

Sows classified as "responders", "non-responders", and "abnormal" in response to suckling reduction treatment were compared in Table 6. A response category × day interaction

was present ($P < 0.001$) for E2, as E2 increased from d 18 to 25 for controls, increased to d 21 then decreased in responders and in non-responders to a lesser extent, while E2 was lowest ($P < 0.05$) in abnormal sows regardless of the time point.

The E2 profile of all 132 sows are represented in either Figure 1 or Figure 2 depending on their response to treatment. Sows ovulating within 7 days of treatment initiation or weaning are depicted in Figure 1, and these E2 profiles generally correspond with ultrasounded sows from farrowing group 2 and 4 that had been classified as controls or responders (Table 6); accordingly, a similar treatment × day interaction ($P < 0.001$) was observed. Among all farrowing groups, the 36 sows failing to ovulate within 7 days of treatment initiation are shown in Figure 2, and the E2 responses shown therein validate the relatively inactive E2 profiles of the ultrasounded sows classified as non-responders or abnormal.

Follicle characteristics did not differ based on response category, but responders developed preovulatory-sized follicles more rapidly ($P < 0.05$) than controls and non-responders, and the slowest ($P < 0.05$) rate of follicle development was in the 6 abnormal sows. Control sows ovulated the fastest ($P < 0.05$) after follicles > 6 mm were present, and accordingly, reached maximum follicle diameter the fastest ($P < 0.05$) relative to weaning or treatment initiation; whereas non-responders and abnormal sows reached maximum follicle diameter approximately 5 d later ($P < 0.05$) post-farrowing compared to controls and responders. Onset of estrus after treatment initiation or weaning was latest ($P < 0.05$) for non-responders, while abnormal sows remained in estrus the longest ($P < 0.05$). Hence, ovulation was also delayed ($P < 0.05$) in non-responders and abnormal sows relative to controls and responders.

The SEP litters had the greatest ($P < 0.05$) creep feed disappearance both on a litter basis and when adjusted and reported as g/pig/d (Table 7). While not to the magnitude of SEP litters,

pigs in the 24HR treatment also had greater ($P < 0.05$) creep feed use than control, ALT or SW litters. Day 21 pig weights depicted in Table 8 indicate increased ($P < 0.05$) pig BW for controls, reflecting the post-weaning growth check experienced by pigs weaned on d 18 in ALT and SW treatments, and the negative effect of decreased nursing time in SEP and 24HR pigs. Conversely, control pigs were lighter ($P < 0.05$) at d 25, which was 4 d after controls were weaned. Pigs from SW litters were heaviest ($P < 0.01$) on d 28 and similar to control and ALT pigs, were heavier ($P < 0.05$) than SEP and 24HR pigs at d 32. Accordingly, ADG from d 18 to 32 was poorest ($P < 0.05$) in SEP and 24HR pigs. Nonetheless, no differences in BW were found at the end of the nursery phase (d 49) or at marketing (d 170), congruent with the lack of a difference in ADG beyond d 32. Unexpectedly, carcass yield was decreased ($P < 0.05$) in control pigs compared to ALT and 24HR pigs, with SEP and SW pigs intermediate. Moreover, the greatest ($P < 0.05$) lean percentage was in ALT pigs and lowest ($P < 0.05$) in SW pigs, with other treatments similar.

To compare the growth of light- and heavyweight pigs among treatments, the criteria applied to SW and ALT pigs on d 18 were also retrospectively applied to the other treatments and shown in Table 9. Treatment × d 18 BW interactions were present ($P < 0.01$) for every BW and ADG measure except d 170, as lightweight ALT and SW pigs gained more BW than other lightweight pigs until d 32, but this benefit was no longer present at d 170. Overall, pigs lightweight at d 18 remained lighter ($P < 0.001$) to market weight regardless of suckling treatment. During the finishing period (d 49 to 170), the tendency for a treatment × d 18 BW interaction ($P = 0.055$) was driven by similar ADG between initially heavyweight and lightweight pigs in control, ALT, and SW treatments, while initially heavyweight pigs in SEP and 24HR treatments maintained their ADG advantage over lightweight pigs. This corresponds with the tendency for a treatment × d 18 BW interaction ($P = 0.059$) for HCW, as initially

heavyweight SEP and 24HR pigs had heavier HCW than lightweight SEP and 24HR pigs, while HCW were similar for initially light- and heavyweight pigs in the control, ALT, and SW treatments. Moreover, a tendency for a treatment × d 18 BW interaction ($P = 0.073$) was also present for loin depth, as initially lightweight pigs at d 18 had deeper loins than initially heavyweight pigs in ALT, SEP and 24HR treatments, whereas initially heavyweight pigs had deeper loins in the control and SW treatments.

The within litter weight variation as CV is shown in Table 10. Litters where the ALT and SW treatments were applied had decreased ($P < 0.01$) variation on d 21 and d 25, and SW litters continued to have less ($P < 0.05$) within litter BW variation versus controls, SEP, and 24HR litters until d 32. Nonetheless, BW variation within litter was similar at the end of the nursery and at marketing (d 170), with no differences between treatments for the change in CV from d 18 to d 170.

DISCUSSION

The occurrence of lactational estrus is typically prohibited by the suckling intensity of the piglets and the negative energy and/or protein balance of the sow which often occurs due to the metabolic demands of lactation (Quesnel, 2009). The presence of suckling piglets and teat stimulation elicits neuroendocrine reflexes which stimulate the release of endogenous opioid peptides (EOP). The release of EOP suppresses gonadotropin secretion (De Rensis et al., 1993), thereby restricting the accumulation of peripheral luteinizing hormone (LH) and inhibiting LH pulses which are needed to mount a successful preovulatory LH surge leading to ovulation. This period of relative ovarian inactivity changes as lactation progresses, since releasable LH pools are gradually restored (Jones and Stahly, 1999) and the sow develops a greater capacity to mount an LH surge in response to estrogens (Sesti and Britt, 1993). Accordingly, in some contemporary

hyperprolific sow genotypes, sows can escape the lactational inhibition and ovulate before weaning, especially multiparous sows with high feed intake and longer lactations (Gerritsen et al., 2009; Kemp and Soede, 2012). In the present study, serum P4 concentrations revealed that 1 multiparous sow had ovulated prior to treatment allocation on d 18.

While early attempts to induce estrus during lactation yielded inconsistent responses (Crighton, 1970; Thompson et al., 1981; Stevenson and Davis, 1984a), they clearly indicated that reduction of the suckling stimulus using methods such as IS or SW was an important feature of successful lactational estrus induction. Efforts to extend the weaning age and transition to group gestation housing have generated renewed international interest in lactational estrus. Furthermore, recent reports that combined decreased suckling via IS or SW with daily boar exposure have resulted in better estrus responses than previously reported (>90%; Downing et al., 2012, Terry et al., 2013). Moreover, a recent proof of concept study using an ALT treatment combining elements of IS and SW and daily boar exposure, resulted in lactational estrus rates and subsequent fertility similar to controls (Frobose et al., 2013). Another interesting outcome was an observed reduction in litter BW variation during the early nursery period, but pig growth was not followed to market. Nevertheless, concerns around additional labor required to implement the ALT treatment and the impracticality of removing sows to an outside boar limited commercial interest. Consequently, the present experiment was designed to consider methods of suckling reduction which vary in their complexity and level of suckling stimulus reduction. To more efficiently provide boar stimuli, it was agreed that delivering nose-to-nose boar contact to lactating sows inside the farrowing room would be more practical, and would therefore be utilized in the present study.

Once trained to work with the remote-controlled boar cart, mature boars provided a simple, effective means to ensure each sow received 5 min of boar contact daily. The hand-held remote control also allowed 1 handler to maneuver the boar efficiently while simultaneously checking sows for standing estrus response. Although boar exposure for 3 d prior to weaning did not cause any control sows to show estrus during lactation, previous research has shown that boar exposure during late lactation can shorten the WEI and reduce the number of anestrus sows (Walton, 1986). Since the provision of boar exposure daily within the farrowing room prohibited the availability to have control sows without some boar stimulation prior to weaning, this pre-weaning boar exposure could have contributed to high post-weaning fertility in controls. Controls were also at times adjacent to estrus sows, and since proximity to an estrus sow is known to enhance onset to estrus in weaned sows (Pearce and Pearce, 1992), this must also be considered as a potential contributing factor to the high fertility observed for controls in the present study. Also, full boar contact yielded greater lactational estrus response (67 vs. 56%) compared to fence-line exposure alone (Terry et al., 2013; Weaver et al., 2014), and the potential decrease in boar stimulus value in the present study must also be considered.

The suckling reduction methods tested in the present study produced differing effects on sow fertility and litter growth, yet there is a paucity of previous experiments simultaneously comparing more than one suckling reduction method. Litter separation, also known as IS, is arguably the most understood method used to stimulate lactational estrus. Langendijk et al. (2007) and Gerritsen et al. (2008) reviewed the optimal presentation of IS and effects on sow reproductive performance. They reported that up to 90% of sows are likely to show lactational estrus if the following conditions are met: 1) IS should not be initiated until d 18 postpartum, 2) IS should last for at least 10 h/d, 3) during IS, sows should be housed out of sight and sound of

piglets, and 4) some form of boar contact should be provided. These recommendations are consistent with results of some recent experiments (Downing et al., 2011; Downing et al., 2012); but Soede et al. (2012), following these recommendations, found only 23% of primiparous and 68% of multiparous sows in estrus during lactation. This variation may be related to disparity in the amount of primiparous sows across IS experiments as well as variation in genotypes, which Langendijk et al. (2009) demonstrated is an important factor for successful induction of lactational estrus. The application of SEP in the present study was designed to adhere to these conditions, except a novel component of the SEP treatment was the use of a communal area in between 2 SEP sows as the location of the separated piglets, instead of housing the piglets out of sight and sound of the sow. The common area was designed to utilize housing space already available, reduce labor otherwise necessary to completely remove piglets to an external room, and to incorporate a commingling component into SEP. Previous studies have shown benefits to co-mingling prior to weaning including reduced aggression (Weary et al., 2002; Parratt et al., 2006), faster establishment of a dominance hierarchy (D'Eath, 2005), and increased post-weaning weight gain (Weary et al., 2002).

Although SEP sows did not differ significantly from other treatments in the ability to induce lactational estrus, the estrus response was numerically lower (73%) and conception rate (63%) for lactationally-mated SEP sows was below levels consistently observed in commercial herds. Contributing to the lower SEP response were several sows (4 of 19) that responded uncharacteristically to the SEP treatment. These sows initially showed follicle growth and displayed estrus behavior during lactation, but within 7 d of weaning, these sows were again found in estrus. While the exact mechanism behind this observation is unclear, a potential causative factor may have been related to the decision not to house piglets out of sight and

sound. During separation (0600 to 1800 h), and particularly when pigs in other, neighboring litters were nursing, SEP pigs became increasingly restless, active, and vocal. This led to increased perceived stress for both sow and piglets when pigs were reintroduced to sows at 1800 h each day. In the first farrowing group, multiple SEP pigs jumped over the separation panel (~60 cm in height), and wire panels had to be placed over the communal area to prevent additional pigs from escaping. The timing of separation may have played a role as well, and overnight separation should be also be considered as pigs may spend a larger portion of their time budget resting. Finally, the presence of 2 SEP litters cross-suckling for 12 h/d also introduced a foreign piglet component, which may play a role as work in beef cows has demonstrated that the mother-offspring bond is important in the suckling-mediated inhibition of LH secretion (Silveira et al., 1993)

Split-weaning is another method which has been used to reduce the suckling stimulus, although in the past SW was primarily intended to decrease WEI and synchronize post-weaning estrus (Stevenson and Britt, 1981; Cox et al., 1983). Permanently removing a portion of the litter also reduces the lactation demand for nutrients and reduces the catabolism of sow energy and protein stores (Vesseur et al., 1997). Until recently, SW had not been used for lactational estrus induction, although SW is known to accelerate the resumption of ovarian activity (Zak et al., 2008) and can decrease prolactin levels that contribute to gonadotropin suppression prior to weaning (Degenstein et al., 2006). In 2 recent experiments by Terry et al. (2013; 2014), high rates of lactational estrus (83 to 95%) were observed when 3 to 7 pigs were weaned and provided daily fence-line boar exposure. However, decreased subsequent farrowing rate and NBA were reported and another experiment by Kirkwood et al. (2013) only found 48% of sows showing estrus in lactation. In the current experiment, SW yielded the highest rate of lactational estrus

(85%) and conception rate (92%) among suckling reduction treatments. It is worth noting that no SW sows were classified as abnormal, and the 4 SW sows not responding with lactational estrus and ovulation all ovulated normally with a short (3.6 d) WEI. Unfortunately, in the present study, subsequent fertility could not be recorded, as results from Terry et al. (2014) indicated potentially poorer farrowing rate and decreased NBA for SW sows. Taken together, the available information supports using SW and boar exposure to induce high rates of lactational estrus.

The ALT treatment presentation was initially conceived as an adaptation to Britt and Levis (1982), where 2 complete litters were combined and rotated between adjacent sows for 48 h prior to weaning, resulting in reduced WEI. Frobose et al., (2013) reported lactational estrus in 95 and 75% of multi- and primiparous ALT sows, respectively, when ALT was combined with provision of 15 min of fence-line and full boar contact. In the present study, 78% of ALT sows showed lactational estrus and with similar, albeit numerically lower, conception rates compared to controls (78 vs. 97%). The poorer response to ALT compared to Frobose et al. (2013) may be in part attributed to differences in season and different presentation of boar stimuli. Interestingly, 2 ultrasounded ALT sows showed abnormal follicle development and estrus behavior, as they were detected in estrus during lactation and inseminated, but then remained in standing estrus for 6 and 7 d and failed to ovulate until the end to the observed "persistent" estrus. This phenomenon was only recorded in the ALT treatment, but the remaining ALT sows seemed to generally fit the previously described "all or none" response to suckling reduction coined by Stevenson and Davis (1984b).

The fourth suckling reduction method, 24HR, had not been tested previously, but was hypothesized as a means to accelerate the processes necessary to overcome the suckling-induced suppression of LH, thereby allowing for sows to express lactational estrus when accompanied by

daily boar exposure. Based on information provided by W. Hurley (personal communication), initial concerns that 24HR sows would have markedly lower milk yield after 24 h of separation were ameliorated. This is supported by a report from Theil et al. (2005) where piglet BW gain was reduced by approximately 20% once nursing resumed after a 24 h removal. Theoretically, 24HR thus represented a less labor-intensive means to reduce the suckling stimulus, and the effects of 24 h of separation on piglet performance and creep intake was also of interest. While the present results show that 24HR can yield lactational estrus (63%) in a portion of sows, the poor conception rate (60%) for these sows makes 24HR unlikely to warrant additional investigation as a means to consistently induce lactational estrus. Moreover, 4 of the 24HR sows had follicle growth to preovulatory size, but failed to ovulate, either showing follicle regression or returning to estrus within 7 d of weaning.

A portion of the sows that were ultrasounded in the reduced suckling treatments, particularly ALT, SEP, and 24HR, were classified as "non-responders" (n = 11) or "abnormal" (n = 6). These sows were grouped into response categorizes for post hoc comparison against controls and "responder" sows having follicle growth > 6 mm and ovulation within 7 d. Patterns emerged for these classifications, as non-responders primarily differed from responders with delayed follicle development and lower peak E2 levels by d 25. These non-responders were commonly primiparous sows more likely to be in a negative energy balance due to the concurrent demands of growth and lactation (Langendijk et al., 2000; Lucy et al., 2001; Hoving et al., 2011), and consequently less likely to exhibit lactational estrus (Stevenson and Davis, 1984a). Moreover, this experiment took place during the summer months, and due to limited body reserves at farrowing, primiparous sows are the most susceptible to the negative effects of high ambient temperature on lactation feed intake (Hughes 1998).

Where non-responders seemed to fit the "all or none" response, generally showing a normal WEI interval after complete weaning, the 6 sows classified as abnormal did not. As depicted in Table 6, a consistent pattern for the abnormal sows was initial follicle growth to 6 mm at a rate similar to responders. However, these sows then failed to ovulate by either remaining in estrus for an extended time with large preovulatory follicles present, or appearing to ovulate in response to a lactational estrus, but then exhibiting estrus behavior within 7 d of weaning and ovulating in response to the post-weaning estrus. Intriguingly, these abnormal sows consistently had very low E2 levels from d 18 to 25. Among all 5 farrowing groups and including all treatments, sows failing to ovulate within 7 d showed a similarly inactive E2 profile (Figure 2). Since no sows were determined to be cystic, and treatments were initiated after d 18, it is unlikely that these abnormal patterns were a result of insufficient LH stores or pulsatility (Langendijk et al., 2009). The 4 sows exhibiting a post-weaning estrus within 7 d after exhibiting lactational estrus were from SEP and 24HR treatments, and these sows may have experienced similar piglet behaviors and suckling suppression. At this time, it is unclear what led to these abnormal follicle growth and estrus behaviors, but future research should be undertaken to determine how to limit their occurrence.

Although the lactational estrus responses were poorer in SEP and 24HR treatments, a positive outcome was the increased creep feed intake in SEP and 24HR litters. This additional creep intake coincided with decreased pig BW at weaning (d 25) due to limited nursing and potentially decreased milk yield, but SEP and 24HR pigs experienced a less marked post-weaning growth check in these pigs. Other IS treatments have also reported increased creep intake during late lactation, which can help reduce the post-weaning growth check (Kuller et al., 2007a; Berkeveld et al., 2007). A reduced post-weaning growth check via prevention of fasting

can help maintain gut health and function during the peri-weaning period (Pluske et al., 1996). However, most reports agree that this benefit is fleeting and not maintained to market weight (Matte et al., 1992; Kuller et al., 2007b). Based on this data, the additional creep intake and less severe post-weaning growth check induced by litter separation for 12 h/d or a single 24 h period is not sufficient to overcome the decreased pig weaning weight. However, one may consider short-term (1 to 3 d) applications of similar separation/commingling techniques as an alternative.

In a previous report (Frobose et al., 2013), lightweight pigs that rotationally-suckled ALT sows had improved growth to d 32 and this effect reduced litter BW variation compared to controls. In the present study, lightweight pigs in ALT and SW treatments benefited similarly from the additional 7 d of nursing access, but the overall benefit over control pigs was no longer present after d 28. Consistent with the simultaneous benefit to lightweight pigs in ALT and SW treatments and detriment to split-weaned heavyweight pigs previously observed to d 32, ALT and SW litters had reduced BW variation until d 28, after which time no differences in BW variation were detected.

Taken together, the results of this study indicate that the suckling reduction strategies used vary in their ability to induce lactational estrus, with SW and ALT treatments responding similarly to sows conventionally mated post-weaning. Furthermore, 5 min of fence-line boar contact delivered in front of the farrowing crate was a sufficient level of boar stimulus to induce lactational estrus. Subsequent fertility of SW and ALT sows lactationally-mated deserves additional attention, as do the abnormal patterns of follicular development and estrus behavior observed in some sows. Regarding pig performance, SEP and 24HR treatments stimulated creep feed intake and reduced the severity of the post-weaning growth suppression, and lightweight pigs in ALT and SW treatments benefited from the additional nursing access. While these initial

pig growth differences were not detected at market weight, the large variation in d 170 BW and differences in carcass yield and lean percentage among treatments may warrant additional exploration.

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Table 3.1. The effects of suckling reduction strategies on lactational characteristics of boar-exposed lactating sows[1]

Item	Control	ALT	SEP	SW	24HR	Probability, $P <$[2]
Sows, n	26	28	26	27	28	
Parity	2.4±0.3	2.6±0.3	2.7±0.3	2.5±0.3	2.6±0.3	0.937
Piglets suckling, d 18[3]	11.4±0.4	11.2±0.4	11.3±0.4	11.1±0.4	11.3±0.4	0.930
d 18 litter weight, kg	64.8±0.4	64.7±0.4	65.5±0.4	64.2±0.4	65.0±0.4	0.988
Sow BW after farrowing, kg						
d 1	236.1±5.5	244.3±5.2	241.9±5.4	234.8±5.3	236.0±5.2	0.634
d 18	230.7±6.2	234.4±5.8	236.1±6.0	227.4±5.9	228.3±5.9	0.764
d 21	228.9±5.8	231.2±5.5	235.5±5.7	226.7±5.6	227.0±5.5	0.790
d 25	205.3±5.5[a]	231.0±5.2[b]	233.0±5.4[b]	224.1±5.3[b]	225.1±5.2[b]	0.003
Lactation BW change, kg[4]	-7.4±2.3	-13.3±2.2	-9.0±2.2	-10.9±2.2	-10.9±2.2	0.290
Lactation BW change, %[4]	-3.2±0.9	-5.4±0.9	-3.7±0.9	-4.5±0.9	-4.7±0.9	0.334
Sow back fat after farrowing, mm						
d 1	15.9±1.0	16.3±0.9	15.1±0.9	15.7±0.9	15.9±0.9	0.690
d 18	14.5±0.7	15.8±0.7	14.6±0.7	14.3±0.7	14.8±0.7	0.416
d 21	13.7±0.8	14.3±0.8	13.2±0.8	14.2±0.8	13.6±0.8	0.573
d 25	13.1±0.7[a]	15.1±0.7[b]	13.5±0.7[a]	14.5±0.7[ab]	14.0±0.7[ab]	0.054
Lactation backfat change, mm[4]	-2.1±0.7	-1.3±0.6	-1.6±0.6	-1.3±0.6	-2.0±0.6	0.655
Lactation ADFI, kg[5]	4.81±0.33	4.55±0.33	4.85±0.33	4.65±0.33	4.98±0.33	0.334
Total lactation feed intake, kg[5]	108.9±8.9[a]	117.6±8.8[ab]	126.5±8.9[b]	121.7±8.9[b]	130.4±8.9[bc]	0.008

[a, b] Means without a common superscript differ, $P < 0.05$.

[1] A total of 135 sows (PIC 1050) were used across 5 farrowing replicates. Sows were allotted to treatments on d 18 of lactation. Treatments were: Control; ALT (sows placed in adjacent pairs, on d 18 all but the 5 lightest piglets were weaned and remaining piglets combined and alternated between sows at 12 h intervals from d 18 to 25; SEP (piglets separated for 12 h/d from d 18 to 25); Split-wean (SW; all but the 5 lightest piglets weaned on d 18); and 24HR (piglets separated from sows for 24 h on d 18). Controls were weaned at d 21, with all other treatments weaned at d 25.

[2] No treatment × farrowing group interactions were detected ($P > 0.238$).

[3] No differences in pig mortality were detected ($P > 0.886$) between treatments.

[4] Lactation weight and backfat loss were measured from d 0 to 21 for control sows and 0 to 25 for the 4 reduced suckling treatments.

[5] Incorporates feed intake from actual farrowing date for each sow.

Table 3.2. Number of boar-exposed sows showing lactational estrus and ovulation in response to suckling reduction[1]

Item	Control	ALT[2]	SEP	SW	24HR
Sows, n	25	27	26	27	27
Reproductive parameters during lactation					
Lactational estrus between d 18 and d 25	0	21	19	23	17
Ovulated[3]	0	18	15	23	13
Anovulatory	0	3	4	0	4
Reproductive parameters post-weaning					
Post-weaning estrus	25	6	7	4	10
Ovulated[3]	24	5	7	4	10
Anovulatory	0	1	0	0	0

[1] A total of 135 sows (PIC 1050) were used in 5 farrowing groups. Sows were allotted to treatments on d 18 of lactation. Treatments were: Control; ALT (sows placed in adjacent pairs, on d 18 all but the 5 lightest pigs were weaned and remaining pigs combined and alternated between sows at 12 h intervals from d 18 to 25; SEP (pigs separated for 12 h/d from d 18 to 25); Split-wean (SW; all but the 5 lightest pigs weaned on d 18); and 24HR (pigs separated from sows for 24 h on d 18). Controls were weaned at d 21, with all other treatments weaned at d 25.

[2] Based on progesterone concentrations, 1 ALT sow ovulated prior to d 18 post-farrowing and was therefore removed from the analysis.

[3] Serum progesterone > 4.0 ng/mL on d 8 to 12 d post-estrus.

0

Table 3.3. The timing of estrus and conception rates of boar-exposed lactating sows in response to suckling reduction strategies[1]

Item	Control	ALT	SEP	SW	24HR	SEM	Probability, $P <$[2]
Sows, n[3]	25/26	27/28	27/27	27/27	27/28		
Lactating sows inseminated,[4] %	0.0	77.8	73.1	85.2	62.9	0.09	0.318
Day 18 to insemination, d	---	5.0[ab]	4.7[a]	5.5[b]	4.4[a]	0.33	0.036
Conception rate,[4,5] %	---	80.4	62.8	87.9	59.8	0.14	0.133
Sows inseminated post-weaning,[4] %	100.0	22.2	26.9	14.8	37.0	0.09	0.318
Wean to estrus, d	3.5	3.8	4.5	3.6	4.3	0.75	0.131
Day in estrus after farrowing	24.5	24.3	24.6	24.4	25.0	0.66	0.868
All sows conception rate,[4,5] %	96.7[b]	78.3[ab]	75.0[ab]	92.0[b]	66.3[a]	0.08	0.094

[a, b] Means without a common superscript differ, P < 0.05.

[1] A total of 135 sows (PIC 1050) were used in 5 farrowing groups. Sows were allotted to treatments on d 18 of lactation. Treatments were: Control; ALT (sows placed in adjacent pairs, on d 18 all but the 5 lightest pigs were weaned and remaining pigs combined and alternated between sows at 12 h intervals from d 18 to 25; SEP (pigs separated for 12 h/d from d 18 to 25); Split-wean (SW; all but the 5 lightest pigs weaned on d 18); and 24HR (pigs separated from sows for 24 h on d 18). Controls were weaned at d 21, with all other treatments weaned at d 25.

[2] No treatment × farrowing group interactions were detected ($P > 0.082$).

[3] Removed from analysis: 1 control sow died the d of weaning, 1 ALT sow ovulated prior to d 18, and 1 24HR sow with an ulcer who never returned to estrus post-weaning.

[4] χ^2 analysis was conducted using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC) to compare treatment means.

[5] Based on transabdominal ultrasound at 28 to 35 d after insemination.

1

Table 3.4. In boar-exposed lactating sows with daily ultrasound (n = 53), categories of response to suckling reduction with regard to follicular development, estrus, and ovulation[1]

Item	Control	ALT	SEP	SW	24HR
Follicular development < 6 mm within 7 d					
No pre-ovulatory follicles	0/10	1/12	2/12	2/9	1/10
Post-weaning estrus within 7 d	---	1	2	2	1
Follicular development > 6 mm within 7 d					
Ovulation within 7 d after d 18 or weaning	10/10	7/12	5/12	7/9	5/10
No ovulation	0/10	2/12	5/12	0/9	4/10
Regression of follicles[2]	0	0	3	0	2
Persistent estrus[3]	---	2	0	---	0
Lactational estrus and 2nd estrus within 7 d post-weaning[4]	---	0	2	---	2

[1] Transrectal ultrasound (500V, 5.0 MHz; Aloka, Wallingford, CT) was performed once daily from d 17 until ovulation in sows from 2 farrowing groups. Sows were allotted to treatments on d 18 of lactation. Treatments were: Control; ALT (sows placed in adjacent pairs, on d 18 all but the 5 lightest pigs were weaned and remaining pigs combined and alternated between sows at 12 h intervals from d 18 to 25; SEP (pigs separated for 12 h/d from d 18 to 25); Split-wean (SW; all but the 5 lightest pigs weaned on d 18); and 24HR (pigs separated from sows for 24 h on d 18). Controls were weaned at d 21, with all other treatments weaned at d 25.

[2] This group represents sows with follicle growth up to preovulatory size without ovulation and subsequent follicle regression.

[3] Two ALT sows remained in estrus for 6 to 7 d before ovulating.

[4] Four sows subjected to suckling reduction exhibited estrus behavior and appeared to ovulate during lactation, but returned to estrus and ovulated within 7 d post-weaning.

2

Table 3.5. Estradiol response, follicle development and timing of estrus and ovulation for boar-exposed lactating sows ovulating within 7 d after initiating a suckling reduction treatment[1]

Item	Control	ALT	SEP	SW	24HR
Sows, n	10	9	5	7	5
Estradiol-17β,[2,3] pg/mL					
Day 17	10.6±3.5	7.8±3.6	9.2±3.8	9.5±3.7	7.3±3.9
Day 21	13.1±4.8	17.0±4.9	18.2±5.4	16.0±5.2	16.0±5.8
Day 25	22.6±2.6[b]	7.9±2.8[a]	8.9±3.7[a]	8.7±3.1[a]	8.9±3.7[a]
Follicle development[4]					
Initial follicle diameter, mm	4.2±0.3	4.1±0.3	4.2±0.4	4.1±0.4	3.6±0.4
Maximum follicle diameter, mm	7.9±0.4	8.3±0.4	8.4±0.5	8.3±0.5	8.5±0.5
Follicle diameter at ovulation,[5] mm	7.9±0.4	8.0±0.4	8.2±0.5	7.4±0.5	7.8±0.5
Start of suckling reduction/wean to follicle diameter > 6 mm, h	90±18.4[b]	44±18.7[a]	58±20.1[a]	53±19.2[a]	60±20.3[a]
Interval follicle diameter > 6 mm to ovulation, h	19±9.8[a]	117±10.3[b]	101±13.8[b]	103±11.7[b]	125±13.8[b]
Day of max. follicle diameter after d 18 or weaning	2.8±1.3	5.8±1.3	6.0±1.4	5.4±1.4	6.7±1.4
Day of max. follicle diameter after farrowing	23.8±1.3	23.8±1.3	24.0±1.4	23.4±1.4	24.7±1.4
Estrus					
Estrus onset after start of suckling reduction/weaning, h	80±13.2[a]	111±13.7[b]	110±16.2[b]	118±14.8[b]	117±16.8[b]
Duration of estrus, h	54±7.3	63±7.5	68±8.5	50±7.9	70±8.6
Ovulation					
Interval start of suckling reduction/weaning to ovulation, h	109±24.7[a]	163±25.1[b]	160±27.7[b]	158±26.2[b]	187±28.1[b]
Interval from estrus onset to ovulation, h	29±14.0	51±14.4	50±16.6	38±15.4	68±8.5

[a, b] Overall significance set at $P < 0.05$ for individual treatment comparisons. Means without a common superscript differ, $P < 0.05$.

[1] Data collected for sows from 2 farrowing groups (n = 53).

[2] A treatment × day interaction was detected (quadratic, P < 0.001) where estradiol-17β increased from d 18 to 25 in control sows, but increased rapidly to d 21, then decreased in sows ovulating in response to reduced suckling treatments.

[3] There was an increase (quadratic, $P < 0.001$) in estradiol-17β from d 18 to 25.

[4] Daily transrectal ultrasound (500V, 5.0 MHz; Aloka, Wallingford, CT) measurements were collected from d 17 until 7 d post-weaning. Follicle diameter reported as the average of the 3 largest follicles on each ovary.

[5] Time of ovulation was defined as 12 h prior to the ultrasound exam when fewer than 4 preovulatory follicles remained between both ovaries.

Table 3.6. Timing of estrus and ovulation, follicle development and estradiol concentrations for boar-exposed lactating sows categorized according to response to suckling reduction[1]

		Suckling reduction outcome		
Item	Control	Responder	Non-Responder	Abnormal
Sows, n	10	26	11	6
Estradiol-17β, [2,3] pg/mL				
d 17	10.3±2.6^{b}	9.0±2.0^{b}	7.9±2.5^{ab}	2.8±3.2^{a}
d 21	12.6±2.6^{bc}	17.6±2.1^{c}	10.9±2.5^{ab}	3.3±3.2^{a}
d 25	22.9±2.6^{b}	8.0±2.0^{ab}	7.3±2.5^{ab}	3.4±3.2^{a}
Follicle development[4]				
Initial follicle diameter, mm	4.2±0.3	4.1±0.2	4.4±0.3	3.7±0.4
Maximum follicle diameter, mm	7.9±0.5	8.3±0.4	8.1±0.5	7.9±0.5
Follicle diameter at ovulation, mm	7.9±0.5	7.8±0.4	7.6±0.5	7.8±0.5
Start of suckling reduction/wean to follicle diameter >6 mm, h	90±23.1^{b}	53±21.2^{a}	106±22.8^{b}	149±25.9^{c}
Interval follicle diameter >6 mm to ovulation,[5] h	19±13.8^{a}	112±8.6^{b}	185±13.2^{c}	132±17.9^{b}
Day of max. follicle diameter after d 18 or weaning	2.8±1.0^{a}	5.8±0.9^{b}	10.7±0.9^{c}	11.0±1.1^{c}
Day of max. follicle diameter after farrowing	23.8±1.0^{a}	23.8±0.9^{a}	28.7±0.9^{b}	29.0±1.1^{b}
Estrus				
Interval start of suckling reduction/weaning to estrus onset, h	81±10.6^{a}	113±8.5^{b}	252±10.3^{c}	123±13.9^{b}
Duration of estrus,[6] h	55±7.1^{a}	60±5.1^{a}	68±6.7^{a}	91±10.8^{b}
Ovulation				
Interval start of suckling reduction/weaning to ovulation, h	110±18.3^{a}	163±16.3^{b}	292±17.9^{c}	286±21.1^{c}
Interval from estrus onset to ovulation, h	30±11.1^{a}	49±9.0^{a}	41±10.7^{a}	166±14.2^{b}

[a, b] Overall significance set at $P < 0.05$ for individual treatment comparisons. Means without a common superscript differ, $P < 0.05$.

[1] Sows classified according to response within 7 d of initiation of suckling reduction: "responders" showed follicle growth and ovulation, "non-responders" had no follicle growth > 6 mm or ovulation, and "abnormal" sows had follicle growth > 6 mm but no ovulation.

[2] A response category × day interaction was detected ($P < 0.001$) for estradiol-17 β.

[3] Estradiol-17β tended to increase (quadratic, $P < 0.10$) from d 18 to 25.

[4] Daily transrectal ultrasound (500V, 5.0 MHz; Aloka, Wallingford, CT) measurements were collected from d 17 until 7 d postweaning. Follicle diameter reported as the average of the 3 largest follicles on each ovary.

[5] Time of ovulation was defined as 12 h prior to the ultrasound exam when fewer than 4 preovulatory follicles remained between both ovaries.

[6] Two "abnormal" sows were included that were continuously in estrus for 6 and 7 d.

Table 3.7. Effects of suckling reduction and boar exposure on creep feed disappearance[1,2]

Item	Control	ALT	SEP	SW	24HR	Probability, *P*<
Litters, n	26	28	26	27	28	
Weaning age, d	21	18/25	25	18/25	25	
Creep feed disappearance,[3] g/pig/d	13±2.2[a]	17±2.1[a]	34±2.2[c]	13±2.2[a]	26±2.1[b]	0.001
Total creep feed use, kg/litter	0.95±0.24[a]	1.27±0.23[a]	4.13±0.24[c]	1.06±0.23[a]	3.18±0.23[b]	0.001

[a, b] Means without a common superscript differ, $P < 0.05$.

[1] A total of 135 sows (PIC 1050) were used in 5 farrowing groups. Sows were allotted to treatments on d 18 of lactation. Treatments were: Control; ALT (sows placed in adjacent pairs, on d 18 all but the 5 lightest pigs were weaned and remaining pigs combined and alternated between sows at 12 h intervals from d 18 to 25; SEP (pigs separated for 12 h/d from d 18 to 25); Split-wean (SW; all but the 5 lightest pigs weaned on d 18); and 24HR (pigs separated from sows for 24 h on d 18). Controls were weaned at d 21, with all other treatments weaned at d 25.

[2] Creep feed was offered ad libitum from d 14 post-farrowing until weaning in a rotary creep feeder (Rotechna Mini Hopper Pan, Rotechna, SA, Agramount, Spain).

[3] Calculated to adjust for differences in weaning age and suckled litter size.

Table 3.8. Effects of suckling reduction and boar exposure on pig growth to market and carcass characteristics[1]

Item	Control	ALT	SEP	SW	24HR	SEM	Probability, *P*<
no. of litters	10	10	10	12	12		
Pig BW, kg							
d 18	5.77	6.10	6.02	5.93	6.03	0.293	0.930
d 21[2]	6.59[d]	6.40[bc]	6.31[b]	6.48[cd]	6.12[a]	0.057	0.001
d 25[2]	7.01[a]	7.27[b]	7.15[ab]	7.50[b]	7.30[b]	0.087	0.003
d 28[2]	7.45[a]	7.66[ab]	7.48[a]	7.79[b]	7.50[a]	0.099	0.065
d 32[2]	9.02[b]	8.92[b]	8.22[a]	8.96[b]	8.22[a]	0.138	0.001
d 49[2]	17.2	17.1	16.3	17.0	16.6	0.30	0.209
d 170[2]	132.5	130.2	131.0	128.8	129.6	2.09	0.734
Daily gain[2], kg							
d 18 to 25	0.16[a]	0.20[b]	0.18[ab]	0.23[bc]	0.20[b]	0.012	0.003
d 25 to 32	0.28	0.23	0.15	0.21	0.13	0.017	0.001
d 18 to 32	0.23[b]	0.22[b]	0.16[a]	0.22[b]	0.17[a]	0.010	0.001
d 32 to 49	0.48	0.48	0.48	0.47	0.49	0.013	0.722
d 49 to 170	0.95	0.93	0.97	0.92	0.93	0.016	0.272
d 18 to 170[1]	0.83	0.82	0.82	0.81	0.81	0.014	0.734
Carcass data							
HCW,[2] kg	95.9	94.6	96.3	93.3	94.2	1.54	0.583
Yield,[2] %	72.1[a]	72.8[b]	72.2[ab]	72.3[ab]	72.7[b]	0.20	0.090
Loin depth,[3] mm	61.3	62.9	62.1	60.8	61.2	0.96	0.286
Last-rib backfat,[3] mm	20.22	19.30	19.18	20.62	20.47	0.66	0.435
Lean percentage,[3,4] %	52.6[ab]	53.2[b]	53.1[ab]	52.4[a]	52.5[ab]	0.27	0.045

[a, b] Means without a common superscript differ, $P < 0.05$.

[1] A total of 626 pigs (PIC 327 × 1050) originated from 54 litters in 2 farrowing replicates. At weaning, pigs were allotted to nursery pens (7 pigs/pen) by BW and gender within treatment. Finisher pen allocation was balanced for prior treatments. Birth weights averaged 1.50 ± 0.3 kg and were similar ($P > 0.05$) between treatments.

[2] Adjusted with d 18 BW as a covariate.

[3] Adjusted with HCW as a covariate.

[4] Calculated using NPPC (1991) guidelines for lean containing 5% fat. Lean % = 2.83 + [0.469 × (0.4536 × HCW)] – [18.47 × (0.0394 × Fat depth)] + [9.824 × (0.0394 × Loin depth)]/(0.4536 × HCW).

Table 3.9. The interactive effects of suckling reduction and d 18 weight category on pig growth to market and carcass characteristics[1,2]

Sow Trt:	Control		ALT		SEP		SW		24HR			Probability, *P* <		
Item	Heavy	Light	SW	RS	Heavy	Light	SW	LW	Heavy	Light	SEM	Trt × d 18 BW	Trt	d 18 BW
Pigs	68	50	66	50	66	50	79	59	79	59				
Weaning age, d	21	21	18	25	25	25	18	25	25	25				
Pig BW, kg														
d 18	6.39	4.92	6.69	5.22	6.79	5.10	6.29	5.17	6.59	5.19	0.309	0.008	0.934	0.001
d 21	7.16	5.49	6.89	6.09	7.22	5.47	6.48	6.26	6.82	5.39	0.322	0.001	0.922	0.001
d 25	7.54	5.97	7.63	7.12	8.10	6.27	7.23	7.64	8.07	6.46	0.337	0.001	0.595	0.001
d 28	7.99	6.39	8.27	7.19	8.39	6.67	7.78	7.58	8.25	6.70	0.323	0.001	0.752	0.001
d 32	9.65	7.81	9.87	8.04	9.22	7.31	9.29	8.21	9.01	7.38	0.348	0.008	0.369	0.001
d 49	18.3	15.2	18.4	16.0	17.8	15.0	17.3	16.0	18.0	15.1	0.61	0.001	0.901	0.001
d 170	133.6	129.7	132.2	128.9	135.9	126.2	130.5	125.5	134.2	124.0	2.50	0.141	0.650	0.001
Daily gain, kg														
d 18 to 25	0.16	0.16	0.12	0.28	0.17	0.18	0.13	0.36	0.20	0.19	0.014	0.001	0.001	0.001
d 18 to 32	0.23	0.21	0.22	0.20	0.17	0.16	0.21	0.22	0.17	0.16	0.012	0.001	0.001	0.001
d 25 to 32	0.30	0.25	0.32	0.12	0.16	0.15	0.30	0.08	0.13	0.13	0.017	0.001	0.001	0.001
d 32 to 49	0.51	0.44	0.50	0.47	0.51	0.45	0.47	0.46	0.53	0.45	0.018	0.002	0.791	0.001
d 49 to 170	0.95	0.95	0.93	0.94	0.98	0.95	0.93	0.91	0.95	0.91	0.182	0.055	0.146	0.001
d 18 to 170	0.83	0.83	0.82	0.82	0.84	0.81	0.81	0.80	0.83	0.79	0.017	0.172	0.642	0.001
Carcass data														
HCW, kg	95.9	95.8	94.6	94.9	97.8	94.9	93.6	92.7	96.0	91.9	1.77	0.059	0.385	0.001
Yield, %	72.1	72.2	72.9	72.6	72.1	72.4	72.3	72.3	72.8	72.6	0.29	0.826	0.087	0.043
Loin depth,[3] mm	62.5	59.4	62.1	64.0	61.6	62.8	60.8	61.0	60.6	62.1	1.13	0.073	0.441	0.565
Last-rib backfat,[3] mm	20.3	20.1	19.3	19.4	19.0	19.5	21.0	20.1	20.3	20.8	0.79	0.566	0.413	0.901
Lean percentage,[3,4] %	52.8	52.3	53.1	53.4	53.1	53.1	52.2	52.6	52.5	52.6	0.36	0.419	0.120	0.685

[a, b] Means without a common superscript differ, $P < 0.05$.

[1] A total of 626 pigs (PIC 327 × 1050) originated from 54 litters in 2 farrowing replicates. At weaning, pigs were allotted to nursery pens (7 pigs/pen) by BW and gender within treatment. Finisher pen allocation was balanced for prior treatments.

[2] Treatments were: Control; ALT (sows placed in adjacent pairs, on d 18 all but the 5 lightest pigs were weaned and remaining pigs combined and alternated between sows at 12 h intervals from d 18 to 25; SEP (pigs separated for 12 h/d from d 18 to 25); Split-wean (SW; all but the 5 lightest pigs

weaned on d 18); and 24HR (pigs separated from sows for 24 h on d 18). Controls were weaned at d 21, with all other treatments weaned at d 25. Although litters remained intact until weaning, control, SEP, and 24HR pigs are sorted into “Heavy” and “Light” categories using d 18 BW and the criteria applied to ALT and SW litters.

[3] Adjusted with HCW as a covariate.

[4] Calculated using NPPC (1991) guidelines for lean containing 5% fat. Lean % = 2.83 + [0.469 × (0.4536 × HCW)] – [18.47 × (0.0394 × Fat depth)] + [9.824 × (0.0394 × Loin depth)]/(0.4536 × HCW).

Table 3.10. The effects of suckling reduction and boar exposure on pig BW variation within litter[1,2]

Item	Control	ALT	SEP	SW	24HR	SEM	Probability, *P* <
Litters	10	10	10	12	12		
Pigs per litter	11.8	11.6	11.6	11.5	11.5	0.16	0.415
Litter CV, %							
d 18	17.0	16.5	18.3	13.1	15.2	1.51	0.103
d 21[3]	16.2[b]	12.7[a]	15.6[b]	12.3[a]	15.7[b]	0.45	0.001
d 25[3]	14.7[b]	12.4[a]	15.4[b]	12.1[a]	15.1[b]	0.78	0.003
d 28[3]	15.1[b]	14.0[ab]	14.3[b]	12.1[a]	14.4[b]	0.74	0.057
d 32[3]	14.9	16.0	14.5	14.3	14.3	0.87	0.574
d 49[3]	13.4	13.1	12.5	13.1	13.3	0.81	0.944
d 170[3]	7.4	7.7	8.3	7.4	9.1	0.69	0.267
HCW[3]	7.6	8.0	9.2	7.5	8.8	0.70	0.324
CV change, d 18 to 170	-8.5	-8.2	-7.6	-8.5	-6.8	0.69	0.281

[a, b] Means without a common superscript differ, $P < 0.05$.

[1] A total of 626 pigs (PIC 327 × 1050) originated from 54 litters in 2 farrowing replicates. At weaning, pigs were allotted to nursery pens (7 pigs/pen) by BW and gender within treatment. Finisher pen allocation was balanced for prior treatments.

[2] Treatments were: Control; ALT (sows placed in adjacent pairs, on d 18 all but the 5 lightest pigs were weaned and remaining pigs combined and alternated between sows at 12 h intervals from d 18 to 25; SEP (pigs separated for 12 h/d from d 18 to 25); Split-wean (SW; all but the 5 lightest pigs weaned on d 18); and 24HR (pigs separated from sows for 24 h on d 18). Controls were weaned at d 21, with all other treatments weaned at d 25.

[3] Adjusted with d 18 CV as a covariate.

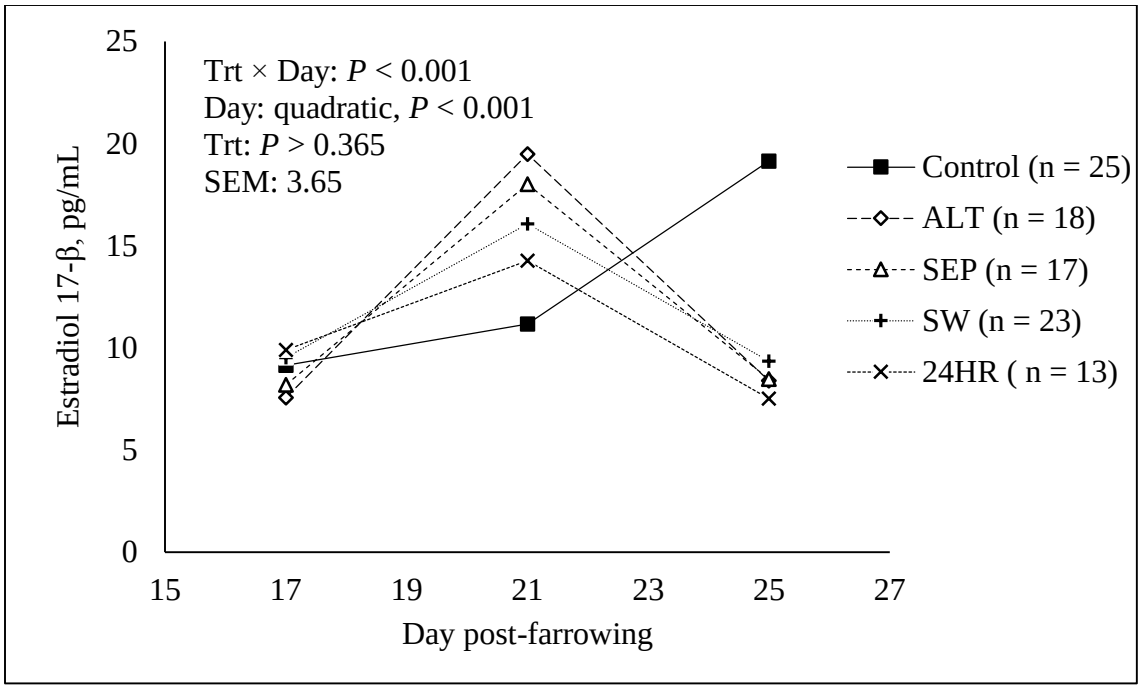

Figure 3.1. Serum estradiol 17-β profile of boar-exposed lactating sows ovulating within 7 d after initiation of suckling reduction or weaning. Treatments were: Control; ALT (sows placed in adjacent pairs, on d 18 all but the 5 lightest pigs were weaned and remaining pigs combined and alternated between sows at 12 h intervals from d 18 to 25; SEP (pigs separated for 12 h/d from d 18 to 25); Split-wean (SW; all but the 5 lightest pigs weaned on d 18); and 24HR (pigs separated from sows for 24 h on d 18). Controls were weaned at d 21, with all other treatments weaned at d 25.

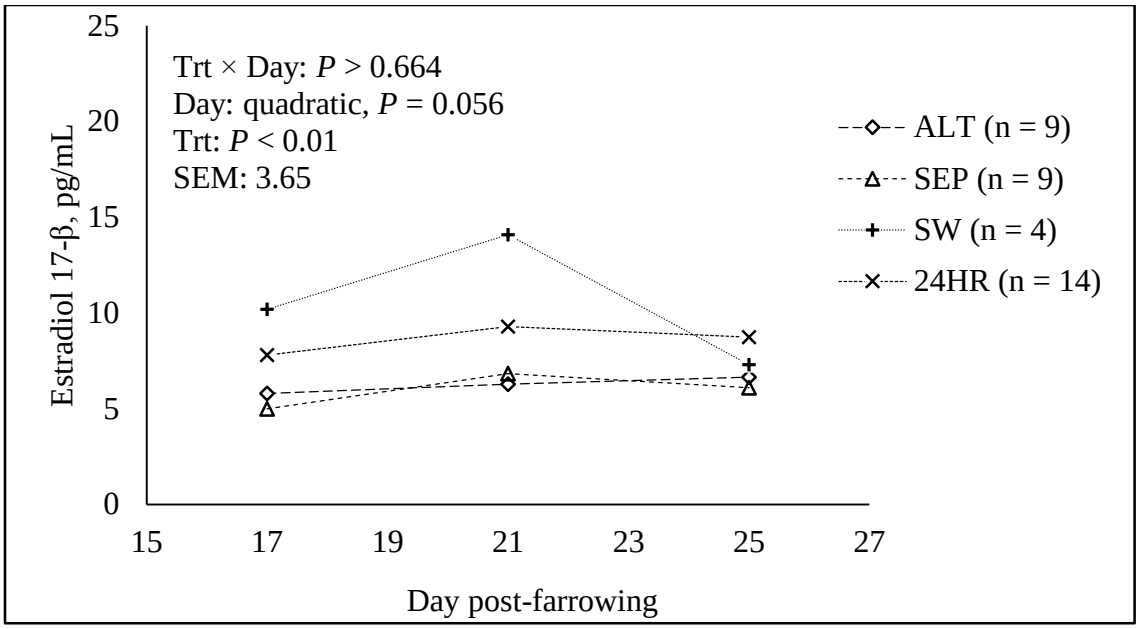

Figure 3.2. Serum estradiol 17-β profile for boar-exposed lactating sows failing to ovulate within 7 d after initiation of suckling reduction. Suckling reduction treatments were: ALT (sows placed in adjacent pairs, on d 18 all but the 5 lightest pigs were weaned and remaining pigs combined and alternated between sows at 12 h intervals from d 18 to 25; SEP (pigs separated for 12 h/d from d 18 to 25); Split-wean (SW; all but the 5 lightest pigs weaned on d 18); and 24HR (pigs separated from sows for 24 h on d 18). Controls were weaned at d 21, with all other treatments weaned at d 25.

Chapter 4 - Effects of Potential Detoxifying Agents on Growth Performance and Deoxynivalenol (DON) Urinary Balance Characteristics of Nursery Pigs Fed DON-Contaminated Wheat

ABSTRACT

Two experiments were conducted to evaluate potential detoxifying agents on the growth of nursery pigs fed deoxynivalenol (DON)-contaminated diets. Naturally DON-contaminated wheat (6 mg/kg) was used to achieve desired DON levels. In a 21 d study, 238 pigs (13.4 ± 1.8 kg BW) were used in a completely randomized design with a 2 × 2 + 1 factorial arrangement. Diets were: 1) Positive control (PC; <0.5 mg/kg DON), 2) PC + 1.0% Product V (Nutriquest LLC, Mason City, IA), 3) Negative control (NC; 4.0 mg/kg DON), 4) NC + 1.0% Product V, and 5) NC + 1.0% sodium metabisulfite (SMB; Samirian Chemicals, Campbell, CA). There were 6 or 7 replicate pens/treatment and 7 pigs/pen. Analyzed DON was decreased by 92% when pelleted with SMB, but otherwise matched formulated levels. Overall, a DON × Product V interaction was observed for ADG ($P < 0.05$) with a tendency for an interaction for ADFI ($P < 0.10$). As anticipated, DON reduced ($P < 0.001$) ADG and ADFI, but the interaction was driven by even poorer growth when Product V was added to NC diets. Pigs fed NC diets had 10% poorer G:F ($P < 0.001$) than PC-fed pigs. Reductions in ADG due to DON were most distinct (50%) during the initial period. Adding SMB to NC diets improved ($P < 0.01$) ADG, ADFI and G:F, and improved ($P < 0.02$) ADG and G:F compared to the PC diet. A urinary balance experiment was conducted using diets 3 to 5 from Exp. 1 to evaluate Product V and SMB on DON urinary metabolism. A 10 d adaptation was followed by a 7 d collection using 24 barrows

in a randomized complete block design. Pigs fed NC + SMB diet had greater urinary output ($P < 0.05$) than pigs fed NC + Product V, with NC pigs intermediate. Daily DON excretion was lowest ($P < 0.05$) in the NC + SMB pigs. However, as a percentage of daily DON intake, NC + SMB fed pigs excreted more DON than they consumed (164%), greater ($P < 0.001$) than pigs fed the NC (59%) or NC + Product V (48%) and indicative of degradation of DONS back to the parent DON molecule. Overall, Product V did not alleviate DON effects on growth nor did it reduce DON absorption and excretion. However, hydrothermally processing DON-contaminated diets with 1.0% SMB restored ADFI and improved G:F. Even so, the urinary balance experiment revealed that some of the converted DON-sulfonate can degrade back to DON under physiological conditions. While additional research is needed to understand the stability of the DON-sulfonate conversion, SMB appears promising to restore performance in pelleted DON-contaminated diets.

INTRODUCTION

Cereal grains are the principal component in swine diets due to the efficiency of cost per calorie provided compared to other ingredients. Nevertheless, fungal infection can occur, and these fungi leave behind secondary metabolites, known as mycotoxins, which have adverse effects on livestock if ingested in sufficient quantities. The bioavailability of some mycotoxins (e.g. aflatoxins or zearalenone) can be reduced by including adsorbent compounds, which reduce mycotoxin uptake and distribution to the blood and target organs (CAST, 2003; EFSA, 2009).

According to a 3-yr global survey (Rodrigues and Naehrer, 2012), the most prevalent (65% of finished feed) mycotoxin in North American feedstuffs is deoxynivalenol (DON), known for its feed intake suppression (Friend et al., 1984) and immunomodulatory effects (Pestka et al., 2004) in pigs when present in diets at over 1 mg/kg. Despite DON's prevalence

and known effects, adsorbent compounds have proven largely ineffective against DON in both in vitro models and in vivo growth studies (Danicke, 2000). Although no DON-detoxifying agents have efficacy claims approved by the U.S. Food and Drug Administration, some products are reported to be of benefit. One such compound is Product V (Nutriquest LLC, Mason City, IA), a proprietary blend of adsorbent clays and preservatives. Since nursery pigs are known for their sensitivity to anti-nutritional factors such as mycotoxins, the objective was to test the growth performance of nursery pigs fed a naturally DON-contaminated diet in the presence or absence of Product V, and to investigate DON absorption and excretion using a urinary balance model. Sodium metabisulfite (SMB; $Na_2S_2O_5$), a known biotransforming agent of DON which, when hydrothermally processed with DON, forms a non-toxic DON-sulfonate adduct (DONS; Beyer et al., 2010) and sulfur dioxide gas, was also incorporated into naturally-contaminated diets to further evaluate SMB's potential for use in DON-contaminated diets.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The nursery and metabolism barns used were both totally enclosed, environmentally controlled, and mechanically ventilated. Sources of naturally DON-contaminated hard red winter (HRW) wheat and uncontaminated HRW wheat were acquired and an initial 17-component mycotoxin screen was performed at North Dakota State University Veterinary Diagnostic Laboratory (NDSU) using a combination of mass spectrometry, ELISA, and HPLC methods. Based on the analyzed DON concentration, an equal amount of high-DON (6.03 mg/kg) or low-DON (0.05 mg/kg DON) wheat was incorporated into experimental diets to achieve desired DON concentrations. Wheat sources were hammer mill ground to approximately 600 μ and each source was homogenously blended to minimize any variation in DON

concentration between diets. Diets were formulated to meet or exceed NRC (2012) requirements and to be identical in nutrient composition apart from DON concentration and the inclusion of detoxifying agents (Table 1). Diets for the growth performance and urinary balance experiment were manufactured simultaneously at the Kansas State University O.H. Kruse Feed Mill. The 5 experimental diets were: 1) Positive control (PC; <0.5 mg/kg DON); 2) PC + 1.0% Product V (a proprietary blend of adsorbent clays and preservatives); 3) Negative control (NC; 4.0 mg/kg DON); 4) NC + 1.0% Product V; and 5) NC + 1.0% SMB ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA). Two large batches using the low- or high-DON wheat were initially mixed to ensure consistency in DON levels. Each individual diet was then manufactured by subdividing the large batches and incorporating the appropriate detoxifying agent or sand at 1.0% of the final diet.

After mixing complete diets for 2 min in a double ribbon mixer, diets were pelleted (CPM Master Model 1000HD; Crawfordsville, IN) at a production rate of 454 kg/h to maintain a minimum conditioner retention time and temperature of 45 s and 82˚C, respectively. Diets were manufactured in numeric order to minimize carryover, with a flush between each diet. Feed mill worker safety was also accounted for since SMB liberates sulfur dioxide under hydrothermal conditions such as in the pelleting process. Although SMB is "generally recognized as safe" by the U. S. Food and Drug Administration, the production of sulfur dioxide by SMB is irritating to the respiratory tract epithelium, causes eye irritation, and can cause severe reactions in asthmatics (Nair and Elmore, 2003). Accordingly, all personnel involved were required to wear respirators and safety goggles during the pelleting process. Samples of each diet were collected both pre- and post-pelleting. Diet samples were stored, frozen, and shipped along with basal

ingredient samples to LABOCEA (Ploufragan, France) for a mycotoxin profile analysis and to Ward Laboratories (Kearney, NE) for nutrient chemical analysis.

Growth Experiment

A total of 238 barrows and gilts (PIC 327 × 1050; initially 13.4 ± 2.5 kg and 40 d of age) were used in a 21 d growth study with 7 replicate pens per treatment and 7 pigs per pen; however, based on limited pen availability, 1 treatment (PC) had 6 replicate pens. Pigs were allotted to pens by initial BW at weaning, and when pigs reached approximately 13 kg, they were reweighed and pen average pig BW was balanced across 1 of 5 treatments in a completely randomized design with a 2 × 2 + 1 factorial arrangement. Deoxynivalenol and Product V inclusion served as main effects with an additional treatment including SMB. Each pen (1.22 × 1.52 m) contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. Pigs were examined daily and feeders were adjusted to maintain approximately 50% pan coverage. Average daily gain, ADFI, and G:F were determined by weighing pigs and measuring feed disappearance on d 7, 14, and 21.

Urinary Balance Experiment

A balance study was also conducted involving pigs individually housed in stainless-steel metabolism cages (1.5 × 0.6 m). Each cage was equipped with a feeder and a nipple drinker for ad libitum access to water. To determine the effects of Product V and SMB on DON urinary excretion and metabolism, only the 3 NC diets from the growth experiment were included. A total of 24 barrows were used over 2 replicate groups (12 pigs per group), with 4 pigs per dietary treatment in each group. Pigs were allotted to treatments in a randomized complete block design based on initial BW and location within the experimental room. Pigs were adapted to the diets and to an amount of feed consumed completely by all pigs (1.4 and 1.6 kg for group 1 and 2,

respectively) and to the metabolism cages during a 10 d adaption period. A 7 d collection period followed where daily feed intake and urinary output was recorded quantitatively. The mean initial BW at the start of the collection period was 42.6 ± 1.7 kg and 51.8 ± 3.5 kg for group 1 and 2, respectively. Feed allocation was divided into 2 equal amounts and given twice daily at 0700 and 1500 h. Due to the low recovery of DON and its primary metabolite de-epoxy-DON (DOM-1) in feces (0.1 to 1.7% of DON intake) in similar studies (Danicke et al., 2007; Danicke et al., 2012), fecal DON and fecal DOM-1 were not analyzed in the present experiment. The separation of feces from urine was achieved by using differently sized screens located beneath the slatted floor of the cage and connected to a funnel and urine collection bottle. Each pig's total daily urine output was frozen and then thawed and homogenously mixed at the end of the collection period. A representative aliquot sample was collected and then frozen before being sent for a full mycotoxin screen at LABOCEA (Ploufragan, France) using liquid chromatography-tandem mass spectrometry with a practical quantitation limit of 0.01 mg/kg.

DON-Sulfonate Quantification

The method used for DONS analysis was described in Beyer et al. (2010). The primary objective of DONS analysis was to confirm that the decreased analyzed DON in the pelleted NC + SMB diet was due to DON structural modification to form DONS, as demonstrated in prior research (Young et al., 1986; Paulick et al., 2015). An automated electrospray ionization-tandem mass spectrometry (ESI-MS/MS) approach was used, and data acquisition and analysis were carried out as in Beyer et al. (2010).

Unfractionated DONS extracts were introduced by continuous infusion into the ESI source on a triple quadrupole MS/MS (4000QTrap, Applied Biosystems, Foster City, CA). An aliquot of 75 µl of extract in methanol/water (3/1 vol/vol) was introduced using an autosampler

(LC Mini PAL, CTC Analytics AG, Zwingen, Switzerland) fitted with the required injection loop for the acquisition time and presented to the ESI needle at 30 µl/min.

A negative neutral loss scan of 80.9 was used to detect the DON-S molecular ion 377 $[M-H]^-$. The ESI-MS/MS parameters used were: DP -80, EP -10, CE-36, CXP -15, electrospray capillary voltage -4500, collision gas pressure 2 (arbitrary units), interface heater on, source temperature (heated nebulizer) 300°C, curtain gas 20 and both ion source gases 45 (arbitrary units). Seventy-five continuum scans were averaged in multiple channel analyzer mode (MCA).

The background of each spectrum was subtracted, the data were smoothed, and peak areas were integrated using Applied Biosystems Analyst software. For both replicate groups of the urinary balance experiment, samples of each diet (n = 3) were analyzed in triplicate. Peak areas of DONS of NC+SMB diet were compared to the peak areas of DONS in NC diet and presented as a ratio.

Statistical Analysis

Data collected from both experiments were analyzed using analysis of variance in the MIXED procedure of SAS, version 9.1 (SAS Inst. Inc., Cary, NC). The growth experiment was a completely randomized design and treatment effects were assessed within each experimental period using pen as the experimental unit. The fixed factors in the model were DON level and the presence or absence of Product V. The pre-planned contrasts in the growth experiment evaluated: 1) interactions between DON and Product V, 2) DON vs. non-contaminated, and 3) the absence or presence of Product V in diets. Finally, 2 pairwise comparison contrasts were used to evaluate the effects of 1) adding SMB to DON-contaminated diets and 2) DON-contaminated diets with SMB versus uncontaminated diets with no detoxifying agents present.

Differences among contrasts evaluated for the growth experiment were considered significant at $P \leq 0.05$ and marginally significant if $P > 0.05$ and $P \leq 0.10$.

The urinary balance experiment was analyzed as a randomized complete block design with individual pig as the experimental unit. Data from the 2 replicates was combined and analyzed for replicate × treatment interactions. Due to lack of a significant interaction, replicate × treatment interaction term was removed from the model with replicate and block within replicate included as random effects in the final model. Differences among treatments in the urinary balance experiment were determined using pairwise comparisons protected with an overall treatment effect of $P < 0.10$ and were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Mycotoxin analyses of the high-DON and low-DON wheat at LABOCEA generally matched initial analyses from NDSU, indicating minimal co-contamination with other mycotoxins (Table 2). However, the ground corn used in all diets contained a low level of DON (0.57 mg/kg) and a high level of fumonisin B1 (FUM; 8.01 mg/kg), which is above cautionary levels for swine. Interactive effects between DON and FUM are well-documented (Grenier et al., 2011; Bracarense et al., 2012) and cannot be ruled out completely, but the low inclusion rate (4%) of FUM-contaminated corn in all experimental diets makes the impact of any interactive effects likely minimal on experimental outcomes. The analyzed concentration of DON in final diets in general matched anticipated levels, with the NC + SMB diet being the only exception (0.35 mg/kg). To reiterate, all 3 NC diets were initially prepared as a single, large batch to ensure consistent DON levels. That large batch was then split and the appropriate detoxifying agent or sand was incorporated prior to pelleting. The decrease in analyzed DON is likely attributed to the formation of 5-fold greater ($P < 0.01$) ratio of DONS present in the NC + SMB diet compared to

the NC alone. Deoxynivalenol-sulfonate is a non-toxic product formed by the reaction between SMB and DON which is amplified by hydrothermal environmental conditions (Danicke et al., 2005), such as those present during pelleting in the present study. The presence of low levels of other toxins in experimental diets is most likely inconsequential, as concentrations were well below cautionary limits for growing swine (Thaler and Reese, 2010). Nutrient analyses for CP, Ca, P, and ash content were consistent across experimental diets (Table 3). The addition of 1.0% Product V increased Fe and Mn levels in the diet by approximately 15 and 60%, respectively. Furthermore, the addition of 1.0% SMB increased dietary S and Na concentrations approximately 2-fold versus other treatments.

Growth Experiment

From d 0 to 7, a 2-way interaction for ADFI was detected where adding Product V worsened ADG and ADFI ($P < 0.05$) by a greater magnitude in DON-contaminated diets than in DON-free diets (Table 4). The presence of DON in diets decreased ADG by 52% ($P < 0.001$), driven by 24% lower ADFI ($P < 0.001$) and 56% poorer feed efficiency ($P < 0.01$). However, the addition of SMB to the NC diet markedly improved ADG ($P < 0.001$) and tended to improve ADFI ($P < 0.10$) versus the NC alone. Nevertheless, from d 0 to 7, the NC + SMB diet still tended to decrease ADFI ($P < 0.10$) versus pigs fed the PC.

From d 7 to 14, no DON × Product V interactions were present. While the previously observed worsening of feed efficiency for NC-fed pigs was not observed, pigs fed NC diets had reduced ADFI ($P < 0.01$) and decreased ADG ($P < 0.01$) relative to pigs fed the PC. Adding Product V to diets had no effect on ADG, ADFI or feed efficiency, but the addition of SMB improved ADG ($P < 0.001$) by 20% compared to the NC, driven primarily by an improvement

($P < 0.001$) in feed efficiency. Pigs fed the NC + SMB diet also exhibited 11% greater feed efficiency ($P < 0.01$) than pigs fed PC diets.

From d 14 to 21, a tendency for a 2-way interaction was detected ($P < 0.10$) for ADG where Product V inclusion increased ADG in PC diets, but worsened ADG in NC diets. Average daily gain was decreased ($P < 0.001$) for pigs fed the NC, again driven by reduced ADFI ($P < 0.001$) but also by poorer feed efficiency ($P < 0.05$). Product V addition tended to worsen feed efficiency ($P < 0.10$), but ADG and ADFI were not affected. Supplementation of SMB in NC diets improved ADG, ADFI, and feed efficiency ($P < 0.001$) vs. NC diets alone by the greatest magnitude during the third week of the experiment. Pigs fed the NC + SMB also had increased ADG ($P < 0.05$) compared to pigs fed the PC, driven by an 11% improvement in G:F ($P < 0.01$).

Overall, a 2-way interaction was observed for ADG and final BW where Product V supplementation worsened ADG and final BW ($P < 0.05$) and tended to worsen ADFI ($P < 0.10$) in NC diets but did not affect performance in PC diets. Feeding 4 mg/kg DON in NC diets decreased ADG (24%; $P < 0.001$) and final BW ($P < 0.001$) over the experimental period, reducing ADFI ($P < 0.001$) by 16% and worsening feed efficiency ($P < 0.001$) by 10%. Supplementing 1.0% SMB in the NC diet improved ADG, ADFI, and G:F ($P < 0.01$) over NC alone by 35, 10, and 19%, respectively, resulting in an improvement ($P < 0.001$) in final BW. Unexpectedly, ADG and final BW of pigs fed the NC + SMB diet surpassed even pigs fed the uncontaminated PC diet ($P < 0.05$), primarily driven by an 11% improvement in feed efficiency ($P < 0.001$).

These results reiterate the extent to which high-DON diets can negatively impact nursery pig growth performance. The present data agrees with Etienne and Wache (2008), who cited a 4.6% decrease in ADFI for every 1 mg/kg of DON in the diet, and Frobose et al. (2015), who

described the feed intake suppression pattern as being the most marked during the initial exposure period and lessening over time. The anorexic effects of DON are most frequently attributed to changes in the metabolism and concentration of brain transmitters such as serotonin in cerebrospinal fluid (Prelusky and Trenholm, 1993; Prelusky, 1994), causing delayed gastric emptying and decreasing small-intestinal motility (Rotter et al., 1996). Moreover, pigs develop conditioned taste aversion to DON-contaminated feedstuffs (Ossenkopp et al., 1994), which is consistent with observations of feed refusal and general anxiety in pigs fed DON (Bergsjo et al., 1993; Danicke et al., 2004a). These effects are more severe in pigs than other species as DON is more rapidly absorbed and distributed to target tissues, and DON clearance from cerebrospinal fluid is slowed (Prelusky et al., 1990).

Previous reports of the impact of DON on feed efficiency have been more variable (Rotter et al., 1996). Long-term exposure to DON-contaminated feed is known to worsen feed efficiency in grow-finish swine (Bergsjo et al., 1993; Danicke et al., 2004b; Patience et al., 2014), but in a series of 4 nursery pig experiments, Frobose et al. (2015) consistently observed depressed feed efficiency only during the initial 3 to 7 d of DON-contaminated diet consumption, consistent with the reduction in G:F observed only during d 0 to 7 in the present growth study. This transitory depression in G:F may be partly attributed to wasted feed from pigs sorting due to taste aversion. Additionally, DON reduces villus height (Bracarense et al., 2012), limiting nutrient absorption, and compromises intestinal barrier function (Van De Walle et al., 2010; Pinton et al., 2012), which may increase maintenance requirements. After this initial decrease, the feed efficiency of pigs fed DON-contaminated diets was generally similar to those fed the PC diet.

In the present study, the addition of Product V at 1.0% in DON-contaminated diets did not alleviate DON's negative effects on nursery pig growth. While Product V did not affect growth when added to the PC diet, intriguingly, when Product V was added to high-DON diets, ADG was suppressed by an additional 11%, mainly driven by 9% lower ADFI. Although the negative DON × Product V interaction was unexpected, some adsorbing agents have been reported to be non-selective in that they may affect the utilization of essential nutrients, such as vitamins and minerals (EFSA, 2009). In fact, a review of 23 pig experiments evaluating potential DON-detoxifying agents revealed that the additives tested were twice as likely to worsen rather than benefit pig ADG (Doll and Danicke, 2003). These observations highlight the importance of using complete factorial designs in studies evaluating mycotoxin detoxifying agents to account for non-specific effects of the feed additive tested. It may also be important to consider that while inexpensive adsorbing agents are regularly incorporated into swine diets as a prophylactic measure against other mycotoxins, such as aflatoxins, DON is actually the most prevalent mycotoxin in North American cereal grains (Rodrigues and Nahrer, 2012). The data herein and the review by Doll and Danicke (2003) indicate that the inclusion of these additives may be just as likely to worsen pig growth rather than improve growth if in fact DON is the primary mycotoxin present in diets.

On the contrary, inactivation of DON using SMB appears promising. Pelleting NC diets with 1.0% SMB restored the DON-associated reduction in ADFI, which agrees with previous research (Frobose et al., 2011) and is most likely associated with the greater than 10-fold reduction in analyzed DON levels due to conversion to DONS. However, pelleting NC diets with SMB also resulted in consistent improvement in feed efficiency throughout the duration of the experiment versus not only the NC (18%) but also compared to pigs fed the uncontaminated PC

diet (11%), suggesting that part of the SMB benefit may be independent of DON-contamination. While the biological mechanism remains unclear, the presently observed feed efficiency benefit was also reported by Danicke et al. (2005), who also fed DON-contaminated wheat hydrothermally-treated with SMB to growing pigs. Furthermore, Burnham et al. (1994) realized G:F benefits when a similar compound, sodium sulfite, was added at 1.0% to traditional or extruded soybean meal and fed to pigs. These reports imply that hydrothermal treatment with sulfites may improve nutrient availability for the animal. Unfortunately, due to lack of additional pen space, a sixth treatment using the PC diet plus SMB could not be added to the present study. This data underscores the need to further investigate SMB as a means to enhance pig growth, regardless of the mycotoxin status of the diet.

The release of sulfur dioxide when pelleting diets containing SMB is a concern for feed mill employees. Acute sulfur dioxide exposure causes irritation to the eyes and respiratory tract (Nair and Elmore, 2003) and therefore may require the use of protective equipment. Despite being classified as "generally recognized as safe" by the U.S. Food and Drug Administration, SMB and other sulfites are known to degrade thiamine (Til et al., 1972) and are therefore excluded from use in foods recognized as significant sources of the vitamin (Nair and Elmore, 2003). Thiamine deficiency requires time to develop in pigs (up to 35 d; Gibson et al., 1987), but is characterized by neurological symptoms and can be fatal if left untreated (Hough et al., 2014). Accordingly, unless supplemental thiamine can be delivered externally (e.g. water or injectable) when feeding SMB-treated feed, opportunities beyond short-term SMB use may be limited. Given these concerns, additional research is necessary to determine the minimum SMB level necessary and acceptable feeding duration to minimize feed processing and thiamine deficiency concerns.

Urinary Balance Experiment

The experimental diets used in the urinary balance experiment were sampled daily within each replicate and a subsample of each was sent for mycotoxin analysis at LABOCEA (Table 5). Analyzed DON concentrations were generally similar to those used in the growth study. Daily feed intake was set by the amount of feed consumed daily by NC fed pigs during the 10 d adaptation period and no differences in feed disappearance were observed between treatments during the collection period. Pigs fed the NC + SMB diet had the greatest urine output during the collection period, being significantly greater ($P < 0.05$) than pigs fed NC + Product V, with NC pigs intermediate. The additional urinary excretion is likely attributed to increased water intake due to the elevated dietary Na when 1.0% SMB was incorporated into the diet (Patience and Zijlstra, 2001).

As calculated from analyzed DON levels, pigs fed NC and NC + Product V treatments consumed a greater amount of DON per d ($P < 0.001$) than pigs fed the NC + SMB diet, since DON conversion to DONS occurred during feed manufacturing when SMB was added prior to pelleting. The DONS analysis confirmed that DON to DONS conversion was over 5-fold greater ($P < 0.01$) when 1.0% SMB was added to NC diets prior to pelleting versus the NC alone and NC + Product V. Although DONS is known to lack the emetic activity of DON (Young et al., 1987), interestingly, the addition of SMB to NC diets did not reduce the incidence of vomiting. In fact, NC + SMB pigs vomited on 10 occasions as compared to 7 and 3 for the NC and NC + Product V treatments, respectively (data not shown). Still, the daily DON urinary excretion was reduced ($P < 0.001$) for NC + SMB fed pigs versus the NC and NC + Product V, and the excretion of the primary metabolite DOM-1 was also less ($P < 0.05$) in the NC + SMB pigs. However, when expressed as a percentage of daily DON intake, pigs fed the NC + SMB diet

excreted more DON than they consumed (164%), which was greater ($P < 0.001$) than pigs fed NC (59%) or the NC + Product V (48%) diet.

For pigs fed the NC + SMB treatment, DON recovery greater than 100% appears to indicate that some of the DONS was degraded to the parent DON. Recent work by Schwartz et al. (2013) revealed that 3 structurally unique forms of DONS can be formed by the reaction of DON with sulfites, dependent on the sulfiting agent and processing conditions present. While DONS-1 and DONS-2 are stable across a broad pH range, DONS-3 can decompose to DON at alkaline pH, such as those in the proximal small intestine. Schwartz-Zimmerman et al. (2014) compared sulfiting agents in a follow-up study and found the predominant form produced by the reaction between DON and SMB to be DONS-3. If the sulfonate formation profile was similar in the present study, this would explain the degradation of a portion of DONS-3 back into DON, which would then be detected as additional DON in the urine. Since the gross DON urine recovery remained only 15% of the DON ingested by pigs fed the NC or NC + Product V diets, the physiological impact from the degradation of DONS back to DON in the digestive tract was likely minimal in the present study. Nevertheless, this degradation pattern is important to consider for future research to potentially enhance the efficacy of the reaction with SMB and lower the dietary concentration of SMB needed to alleviate the effects of DON.

The recovery of DON from pigs fed the NC and NC + Product V matches urinary DON recovery rates in previous work. For example, the urinary recovery of ingested DON was 50 to 63% in Friend et al. (1986) and 42 to 72% in Danicke et al. (2004a). Since urine is the main DON absorption and excretion route, if Product V was able to decrease the uptake of DON, urinary DON excretion would also be decreased. However, in the present study, DON recovery was similar between pigs fed NC or NC + Product V diets, and the lack of a Product V response

in urinary metabolism is congruent with the lack of the growth benefit to Product V. Since pigs have limited ability to de-epoxidate DON other than via microbial fermentation in the hindgut, recovery of urinary DOM-1 was minimal (0.2 to 0.9% of DON intake) but consistent with previous work (0 to 1.1%; Danicke et al. 2004a).

In summary, feeding diets contaminated with 4 mg/kg DON to nursery pigs reduces nursery pig growth, most severely during the initial exposure period and primarily via feed intake suppression. The addition of Product V did not alleviate the DON-associated effects on pig growth nor did it reduce DON absorption and urinary excretion compared to pigs fed DON-contaminated diets alone. However, treating DON-contaminated diets with 1.0% SMB restored feed intake and improved feed efficiency markedly. Even so, the urinary balance experiment revealed that a portion of the converted DONS can be degraded back to DON under physiological conditions. While questions remain surrounding processing methods and long-term supplementation effects of SMB, but this research demonstrates that pelleting DON-contaminated diets with SMB can alleviate DON effects on growth. Additional research is also needed to evaluate the effect of sodium metabisulfite on feed efficiency in uncontaminated diets.

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Table 4.1. Composition of experimental diets, (as-fed basis)

Item	Positive control (PC)	PC + 1.0% Product V[1]	Negative control (NC)	NC + 1.0% Product V	NC + 1.0% SMB[2]
Uncontaminated hard red winter (HRW) wheat	67.00	67.00	---	---	---
Deoxynivalenol-contaminated HRW wheat, 6 mg/kg[3]	---	---	67.00	67.00	67.00
Soybean meal, 46.5% CP	24.16	24.16	24.16	24.16	24.16
Corn	4.23	4.23	4.23	4.23	4.23
Limestone	1.40	1.40	1.40	1.40	1.40
Monocalcium phosphate, 21% P	0.60	0.60	0.60	0.60	0.60
Salt	0.35	0.35	0.35	0.35	0.35
L-Lysine-HCl	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.15	0.15	0.15	0.15	0.15
L-Threonine	0.20	0.20	0.20	0.20	0.20
Vitamin premix[4]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[5]	0.15	0.15	0.15	0.15	0.15
Phytase[6]	0.02	0.02	0.02	0.02	0.02
Product V	---	1.00	---	1.00	---
Sodium metabisulfite	---	---	---	---	1.00
Sand	1.00	---	1.00	---	---
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
SID[7] amino acids, %					
Lys	1.28	1.28	1.28	1.28	1.28
Ile:Lys	59	59	59	59	59
Leu:Lys	103	103	103	103	103
Met:Lys	33.4	33.4	33.4	33.4	33.4
Met & Cys:Lys	57.6	57.6	57.6	57.6	57.6
Thr:Lys	62.7	62.7	62.7	62.7	62.7
Trp:Lys	18.4	18.4	18.4	18.4	18.4
Val:Lys	63.9	63.9	63.9	63.9	63.9
Total Lys, %	1.41	1.41	1.41	1.41	1.41
ME, kcal/kg	3,131	3,131	3,131	3,131	3,131
SID Lys:ME, g/Mcal	4.09	4.09	4.09	4.09	4.09
CP, %	20.8	20.8	20.8	20.8	20.8
Ca, %	0.72	0.72	0.72	0.72	0.72
P, %	0.61	0.61	0.61	0.61	0.61
Available P, %	0.42	0.42	0.42	0.42	0.42

[1] A proprietary combination of adsorbent clays and preservatives (Nutriquest LLC, Mason City, IA).

[2] Sodium metabisulfite ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA).
[3] Basal ingredient sample sent to the North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) for a full 17-component toxin screen. Samples were analyzed using a variety of mass spectrometry, ELISA, and HPLC methods with a practical quantitation limit of 0.5 mg/kg.
[4] Provided per kilogram of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D_3; 17,637 IU vitamin E; 1,764 mg vitamin K; 3,307 mg riboflavin; 11,023 mg pantothenic acid; 19,841 mg niacin; and 15.4 mg vitamin B_{12}.
[5] Provided per kilogram of premix: 22.0 g Mn from manganese oxide; 73.4 g Fe from iron sulfate; 73.4 g Zn from zinc sulfate; 11.0 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.
[6] HiPhos 2700 (DSM Nutritional Products LLC, Parsippany, NJ, USA) contains 2,708,400 phytase units/kg premix.
[7] Standardized ileal digestible.

Table 4.2. Mycotoxin analysis of basal ingredients and experimental diets, (as-fed basis)[1, 2]

	Basal ingredients			Experimental diets[3]				
Item	Ground corn	High DON HRW wheat[4]	Low DON HRW wheat	Positive Control (PC)	PC + 1.0% Product V[5]	Negative Control (NC)	NC + 1.0% Product V	NC + 1.0% SMB[6]
Mycotoxin, mg/kg								
Deoxynivalenol (DON)	0.57	5.70	0.05	0.04	0.06	4.10	4.23	0.35
De-epoxy-DON	---	0.02	---	---	---	0.02	0.02	---
15-Acetyl DON	0.05	0.17	---	---	---	0.11	0.13	0.04
3-Acetyl DON	0.01	0.06	---	---	---	0.03	0.03	----
Zearalenone	0.10	0.02	---	---	---	0.01	0.03	0.03
Fumonisin B_1	8.01	0.27	0.38	0.93	0.59	0.63	0.70	0.67
Fumonisin B_2	1.05	0.09	0.13	0.28	0.15	0.15	0.17	0.20
Fumonisin B_3	0.66	0.03	0.05	0.31	0.16	0.23	0.20	0.18
Monoliformine	0.26	---	---	---	---	---	---	---
Ergot alkaloids[7]	---	0.20	---	---	---	0.16	0.15	0.13

[1] A sample was collected after dietary ingredients were mixed into the batch, but prior to the conditioning and pelleting process.

[2] Basal ingredient and experimental diet samples were sent to LABOCEA in Ploufragan, France for a 40 component toxin screen. Samples were analyzed using liquid chromatography-tandem mass spectrometry with a practical quantitation limit of 0.01 mg/kg.

[3] Positive control diets formulated to contain <0.5 mg/kg DON and all remaining diets formulated to contain 4.0 mg/kg DON. All diets were pelleted at 82˚C with a minimum conditioner retention time of 45 s.

[4] Hard red winter (HRW) wheat analyzed for deoxynivalenol (DON) concentration (6.0 mg/kg) prior to diet formulation.

[5] A proprietary blend of absorbent clays and preservatives (Nutriquest LLC, Mason City, IA).

[6] Sodium metabisulfite ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA).

[7] Reported as the sum of the ergot alkaloid compounds ergocornin, ergocristin, ergocryptin, ergometrin, ergosin, and ergotamine.

Table 4.3. Chemical analysis of diets, as-fed basis[1]

Item	Positive control (PC)	PC + 1.0% Product V[2]	Negative control (NC)	NC + 1.0% Product V	NC + 1.0% SMB[3]
DM, %	89.59	89.23	89.55	89.71	89.16
CP, %	22.5	22.4	22.0	22.4	22.2
Ca, %	0.80	0.80	0.82	0.77	0.76
P, %	0.54	0.51	0.55	0.57	0.58
S, %	0.23	0.24	0.24	0.24	0.46
Na, %	0.12	0.14	0.12	0.15	0.32
K, %	0.85	0.83	0.85	0.92	0.92
Mg, %	0.17	0.17	0.17	0.19	0.18
Zn, mg/kg	106	92	127	107	109
Fe, mg/kg	282	320	270	314	233
Mn, mg/kg	63	106	65	102	68
Cu, mg/kg	20	22	20	18	23
Ash, %	5.3	5.0	5.3	5.2	5.1

[1] Dietary samples were collected post-pelleting and sent for chemical analysis at Ward Laboratories (Kearney, NE).

[2] A proprietary blend of absorbent clays and preservatives (Nutriquest LLC, Mason City, IA).

[3] Sodium metabisulfite ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA).

Table 4.4. Effects of potential detoxifying agents on growth performance of nursery pigs fed deoxynivalenol (DON)-contaminated wheat[1]

		Positive control (PC)[2]		Negative control (NC; 4.0 mg/kg DON)[2]				Probability, $P <$[3]				
Item	Detoxifying agent:	None	1.0% Product V[4]	None	1.0% Product V	1.0% SMB[5]	SEM	DON × Product V	DON	Product V	SMB vs. PC	SMB vs. NC
d 0 to 7												
ADG, g		380	375	233	151	403	17.2	0.024	0.001	0.012	0.324	0.001
ADFI, g		644	643	535	439	588	20.5	0.022	0.001	0.019	0.054	0.055
G:F		0.590	0.583	0.440	0.335	0.686	0.029	0.081	0.001	0.045	0.020	0.001
d 7 to 14												
ADG, g		534	526	483	454	578	19.7	0.596	0.003	0.326	0.119	0.001
ADFI, g		832	837	762	706	814	32.0	0.320	0.003	0.414	0.696	0.221
G:F		0.648	0.628	0.633	0.646	0.710	0.015	0.271	0.913	0.813	0.006	0.001
d 14 to 21												
ADG, g		582	632	500	484	647	19.9	0.091	0.001	0.364	0.023	0.001
ADFI, g		921	936	812	772	912	19.5	0.144	0.001	0.511	0.738	0.001
G:F		0.632	0.674	0.616	0.627	0.710	0.016	0.309	0.047	0.091	0.001	0.001
d 0 to 21												
ADG, g		498	510	404	363	543	13.2	0.045	0.001	0.257	0.020	0.001
ADFI, g		798	805	702	639	772	18.3	0.056	0.001	0.113	0.291	0.007
G:F		0.625	0.634	0.576	0.567	0.704	0.011	0.429	0.001	0.987	0.001	0.001
Pig BW, kg												
d 0		13.4	13.4	13.4	13.4	13.4	0.14	0.999	0.966	0.968	0.999	0.976
d 7		16.1	16.1	15.1	14.5	16.3	0.15	0.066	0.001	0.043	0.429	0.001
d 14		19.8	20.2	18.6	17.7	20.3	0.27	0.020	0.001	0.256	0.213	0.001
d 21		23.9	24.2	22.1	21.1	24.8	0.30	0.022	0.001	0.237	0.027	0.001

[1] A total of 238 barrows and gilts (PIC 327 × 1050; initially 13.4 ± 1.8 kg BW and 42 d of age) were used in a 21 d experiment with 6 or 7 replicate pens per treatment and 7 pigs per pen. All diets were fed in pelleted form.

[2] Positive control (PC) and negative control (NC) diets formulated to contain <0.5 mg/kg and 4.0 mg/kg DON, respectively.

[3] Each contrast compared the following treatments: 1) "DON × Product V" evaluated the 2-way interaction between DON and adding 1.0% Product V; 2) "DON" compared PC to NC, excluding only the sodium metabisulfite (SMB) treatment; 3) "Product V" compared diets with Product V (2 and 4) to diets without (Diets 1 and 3); and 4) "SMB vs. PC" and "SMB vs. NC" compared the NC diet with 1.0% SMB to pigs fed the NC or PC diets without detoxifying agents, respectively.

[4] A proprietary blend of absorbent clays and preservatives (Nutriquest LLC, Mason City, IA).

[5] Sodium metabisulfite ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA).

Table 4.5. Urinary excretion of deoxynivalenol (DON), DON-sulfonate (DONS) and the metabolite de-epoxy-DON (DOM-1) in pigs fed DON-contaminated diets with or without potential detoxifying agents[1]

	Negative control (4.0 mg/kg DON)			
Item Detoxifying agent:	None	1.0% Product V[2]	1.0% SMB[3]	SEM
Analyzed DON, mg/kg[4]	4.28	4.63	0.22	
DONS relative to NC[5]	1.00	0.73	5.67	1.318
ADFI, kg	1.46	1.46	1.47	0.045
Urine output, L	20.5^{ab}	18.2^{a}	26.3^{b}	2.16
DON consumption, mg/d	6.21^{b}	6.79^{b}	0.32^{a}	0.223
Excretion in urine, mg/d				
DON	3.65^{b}	3.29^{b}	0.52^{a}	0.164
DOM-1	0.54^{b}	0.39^{ab}	0.18^{a}	0.103
Excretion in urine [% of DON intake]				
DON	58.7^{a}	48.2^{a}	164.4^{b}	6.80
DOM-1	0.24^{a}	0.21^{a}	0.87^{b}	0.037

[1] A total of 24 barrows (PIC 327 × 1050; 42.5 ± 1.7 kg and 51.8 ± 3.5 kg at the onset of the collection period for replicate 1 and 2, respectively) over 2 replicate groups (n = 12) were used in a 17 d experiment with 8 pigs per treatment. The collection period (d 11 to 17) is shown above. All diets were fed in pelleted form.

[2] A proprietary blend of absorbent clays and preservatives (Nutriquest LLC, Mason City, IA).

[3] Sodium metabisulfite ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA).

[4] Analyzed at LABOCEA (Ploufragan, France) using liquid chromatography-tandem mass spectrometry with a practical quantitation limit of 0.01 mg/kg. The average of 2 replicate groups is reported.

[5]Analyzed at the Kansas State University Lipidomics Laboratory using liquid chromatography-tandem mass spectrometry. Peak areas of DONS in the NC + SMB diet were compared to the peak areas of DONS in the NC diet and presented as a ratio.

[a,b] Means without a common superscript differ, $P < 0.05$.

Chapter 5 - The Progression of Deoxynivalenol-Induced Growth Suppression in Nursery Pigs and the Potential of an Algae-Modified Montmorillonite Clay to Mitigate These Effects

ABSTRACT

Two experiments were conducted to characterize the progression of deoxynivalenol (DON)-induced growth suppression and to investigate algae-modified montmorillonite clay (AMMC) as a means to alleviate the effects of DON in nursery pigs. In both experiments, naturally DON-contaminated wheat was used to produce diets with desired DON levels. In Exp. 1, 280 barrows and gilts (10.0 ± 0.2 kg BW) were used in a 28 d experiment arranged in a 2 × 2 + 1 factorial design with 8 replicates per treatment. The 5 treatments consisted of 2 positive control diets not containing DON with or without 0 or 0.50% AMMC and 3 negative control diets with 5 mg/kg of DON and containing 0, 0.25%, or 0.50% AMMC. No DON × AMMC interactions were observed. Overall, pigs fed DON had decreased ($P < 0.001$) ADG and final BW regardless of AMMC addition. Feeding DON-contaminated diets elicited the most severe depression ($P < 0.001$) in ADFI and G:F from d 0 to 3, remaining poorer overall ($P < 0.01$) but lessening in severity as exposure time increased. Pigs fed DON diets had greater ($P < 0.05$) within pen BW variation (CV) on d 28. Although the addition of 0.50% AMMC to diets restored ($P < 0.05$) ADFI from d 14 to 21, no other differences were observed for AMMC inclusion. In Exp. 2, 360 barrows (11.4 ± 0.2 kg BW) were used in a 21 d experiment with 9 dietary treatments arranged in a 3 × 3 factorial design with DON and AMMC inclusion as main effects. There were 8 replicate pens per treatment. Treatments consisted of 3 positive control diets without DON, 3 low DON negative control (1.5 mg/kg DON) diets, and 3 high DON negative

control (3 mg/kg DON) diets with 0, 0.17%, or 0.50% AMMC incorporated at each DON level. No DON × AMMC interactions were observed. As DON level increased, ADG and final BW decreased (quadratic, $P < 0.05$), driven by decreased (quadratic, $P < 0.01$) ADFI and poorer (quadratic; $P < 0.05$) G:F. At both 1.5 and 3 mg/kg DON, reductions in ADG were most marked from d 0 to 7 (15 to 22% lower) and least distinct from d 14 to 21 (5 to 6% lower). Incorporating AMMC at increasing levels had no effect on ADG, ADFI, G:F, or final BW. Overall, these experiments reinforce DON effects on feed intake but also indicate that DON effects on G:F may be more severe than previously thought. Furthermore, some pigs appear to develop tolerance to DON, as effects on ADFI and G:F lessen over time. However, the addition of AMMC did not offset the deleterious effects of DON.

INTRODUCTION

Deoxynivalenol (DON) is a member of the Type B trichothecenes, which are potent inhibitors of protein synthesis (Rotter et al., 1996). Primarily produced by Fusarium fungi, trichothecenes proliferate in cereal grains when flowering coincides with temperate, wet conditions (CAST, 2003). According to a global survey, DON is the most common mycotoxin in North American feedstuffs, present in 75% of samples at an average of 1.3 mg/kg (Rodrigues and Naehrer, 2012).

Among farm animals, pigs are the most sensitive to DON (Eriksen and Petterson, 2004). While vomiting occurs at high concentrations (Forsyth et al., 1977; Pestka et al., 1987), most reports agree that realistic DON levels (1 to 5 mg/kg) primarily decrease feed intake (Friend et al., 1984; Patience et al., 2014). Deoxynivalenol also reduces intestinal absorption (Grenier and Applegate, 2013) and both stimulates and suppresses the immune system (Rotter et al., 1996; Pestka et al., 2004). The severity seems to be dose-dependent and fluctuations may be related to

contradictory feed efficiency effects (Etienne and Wache, 2008). Some reports have observed DON effects lessening over time (Friend et al., 1982; Pollman et al., 1985), but this phenomenon is not well-characterized.

Since environmental conditions dictate DON growth, various methods have been tested to detoxify DON prior to feeding, and feed additives appear to be the most practical (Dänicke, 2002; Awad et al., 2010). Clay minerals used successfully against polar toxins such as aflatoxins have poor DON adsorption (Ramos et al., 1996; EFSA, 2009). Nevertheless, a modified montmorillonite clay (AMMC; Olmix S. A., Brehan, France) has been developed using algal polysaccharides which enhance the DON adsorptive capacity (Havenaar and Demais, 2006). Therefore, the objectives of the present research were to further characterize the progression of DON-induced suppression of growth and to investigate AMMC as a means to alleviate the effects of DON in nursery pigs.

MATERIALS AND METHODS

All experimental procedures and animal care were approved by the Kansas State Institutional Animal Care and Use Committee. In both experiments, diets were corn-soybean meal-based, and a source of both low-DON and naturally DON-contaminated hard red winter wheat was provided by Olmix N.A. (Black River Falls, WI). To maintain consistency and ensure that diets contained the desired level of DON, basal ingredients (corn and the 2 wheat sources) were analyzed for mycotoxin concentration at North Dakota State University Veterinary Diagnostic Laboratory (NDSU; Fargo, ND) prior to diet formulation and incorporated into test diets to achieve desired DON concentrations. At the manufacturer's request, the DON-contaminated wheat was also analyzed for mycotoxin content at LABOCEA (Ploufragan, France). Due to concerns that high-DON wheat may also have a different amino acid profile than

low-DON wheat, both were analyzed for amino acid content at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO), and diet formulation was adjusted to account for the differences. Diets were formulated to meet or exceed all nutrient requirement estimates (NRC, 2012). As recommended by Döll and Dänicke (2003), in order to evaluate both the specific and unspecific effects of the AMMC feed additive, complete factorial designs were used in both experiments. The AMMC product is made up primarily of montmorillonite and 10 to 20% algae. According to a chemical analysis conducted at Dairyland Laboratories (St. Cloud, MN), AMMC contained 6.27% CP, 4.5% sugar, 4.3% Ca, 0.94% Na, 0.90% Cl, 0.17% P and 1.06% K.

Experiment 1

A total of 280 barrows and gilts (PIC 327 × 1050; Hendersonville, TN) were used in a 28 d experiment to determine the effects of DON and AMMC on nursery pig growth. Pigs were initially 10.0 ± 0.2 kg BW and 35 d of age and there were 8 replicate pens per treatment with 7 pigs in each pen. At weaning, pigs were allotted to pens by initial BW, individual variation in BW, and gender. Pigs were fed a common commercial starter diet for 7 d, at which time they were reweighed and pens were assigned to 1 of 5 treatments in a completely randomized design. Treatments were arranged in a 2 × 2 + 1 factorial with DON and AMMC inclusion as main effects. Treatments consisted of 2 positive control diets (PC; <0.5 mg/kg DON), containing either 0 or 0.50% AMMC and 3 negative control diets (NC) formulated to contain 5 mg/kg DON and either 0, 0.25%, or 0.50% AMMC. Apart from the inclusion of DON and AMMC, diets were formulated to be identical in nutrient composition.

Diets were manufactured at the Kansas State University Grain Science Feed Mill. While the stability of AMMC under pelleting conditions is unknown, due to concerns of ingredient

segregation, diets were pelleted. A naturally contaminated source of high-DON wheat (10.7 mg/kg DON) was used to provide diets with 5 mg/kg DON. Following final diet manufacturing, diet samples were sent to NDSU for mycotoxin analysis. Only mycotoxins detected above quantitative limits in at least one of the experimental diets were reported. Final diets were also sent to the University of Missouri for nutrient analysis.

The trial was conducted at the K-State Swine Teaching and Research Center in Manhattan, KS. Each pen (1.22 × 1.52 m) contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. Pigs were examined daily and feeders were adjusted to maintain approximately 50% pan coverage. Average daily gain, ADFI, and feed efficiency were determined by weighing pigs and measuring feed disappearance on d 0, 3, 7, 14, 21, and 28. Since pens were initially balanced for within-pen weight variation, pen CV was also calculated at d 28 to evaluate the effect of DON on pig body weight variation.

Experiment 2

A total of 360 barrows (Line 1050; PIC, Hendersonville, TN; initially 11.4 ± 0.2 kg and 45 d of age) were used in a 21 d experiment to further characterize the effects of DON and AMMC on nursery pig growth. Pigs were shipped to the facility immediately post-weaning and placed in 2 identical nurseries, each containing 40 pens. Upon arrival, pigs were allotted to pens by BW and fed a common commercial diet for the first 24 d. After pigs reached approximately 12 kg, pens were randomly assigned to 1 of 9 dietary treatments. There were 5 pigs per pen and 8 replicate pens per treatment.

Dietary treatments were arranged in a 3 × 3 factorial design with DON and AMMC inclusion as main effects. Treatments consisted of 3 positive control (PC) diets without DON, 3 low negative control (Low NC; 1.5 mg/kg DON) diets, and 3 high negative control (High NC; 3

mg/kg DON) diets with 0, 0.17%, or 0.50% AMMC incorporated at each level of DON. Diets were manufactured in meal form at the Kansas State University O. H. Kruse Feed Mill in Manhattan, KS.

The 0.17% AMMC inclusion rate was chosen to reflect manufacturer-recommended feeding level and the 0.50% inclusion was added to test the ingredient at concentrations known to be effective when similar absorptive clays are added to aflatoxin-contaminated grains (Schell et al., 1993). The AMMC was added at the expense of corn in diet formulation. Diets exceeded NRC (2012) nutrient requirements, and apart from the inclusion of DON and AMMC were formulated to be identical in nutrient composition.

Because of a concern that pelleting may have impacted the efficacy of AMMC in Exp. 1, diets were manufactured in meal form at the Kansas State University O. H. Kruse Feed Mill in Manhattan, KS in Exp. 2. A naturally contaminated source of high-DON wheat (6.0 mg/kg DON) was used to provide diets with desired DON concentrations. Following final diet manufacturing, diet samples were sent to NDSU for mycotoxin analysis. Only mycotoxins detected above quantitative limits in at least one of the experimental diets were reported. Final diets were also sent to the University of Missouri for nutrient analysis.

This experiment was conducted at the Kansas State University Segregated Early Weaning Research Facility in Manhattan, KS. Each pen (1.22 × 1.22 m) contained a 4-hole dry self-feeder and 1-cup waterer to provide ad libitum access to feed and water. Pigs were examined daily and feeders were adjusted to maintain approximately 50% pan coverage. Average daily gain, ADFI, and G:F were determined by weighing pigs and measuring feed disappearance on d 0, 3, 7, 14, and 21.

Mycotoxin Analysis

In both experiments, samples of the basal ingredients (corn and 2 wheat sources) and final diets were sent to NDSU for an 18-component mycotoxin analysis. The analysis for trichothecene mycotoxins (DON, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, nivalenol, and T-2 toxin) along with zearalenone and zearalenol was conducted according to a modified version of Groves et al. (1999) using gas chromatography coupled with mass spectrometry. Aflatoxins and fumonisins were analyzed by HPLC. Samples were tested on an as-fed basis, and the practical quantitation limit for trichothecenes was 0.50 mg/kg, while the detection limits were 2.0 mg/kg for fumonisins and 20 μg/kg for aflatoxins. In both studies, the high-DON wheat was also sent to LABOCEA where a 43-component toxin screen was performed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) techniques. For all toxins, the minimum detection limit at LABOCEA was 10 μg/kg feed.

Statistical Analysis

For both experiments, results were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). The fixed factors in the models included DON level and AMMC inclusion. For Exp. 1, differences were evaluated using pre-planned contrasts which included: 1) the 2-way interaction evaluating the effect of AMMC inclusion at 0.50% compared to none in the positive and negative control diets, 2) pigs fed positive vs negative control diets regardless of AMMC inclusion, 3) The addition of AMMC (0 vs. 0.50%) in both PC and NC diets, and 4) the linear effects of AMMC inclusion within NC diets alone.

In Exp. 2, the main effects of DON level (0, 1.5 or 3.0 mg/kg) and AMMC inclusion (0, 0.17, or 0.50%) and their 2-way interactions served as fixed effects and barn as a random effect

in the model. Preplanned linear and quadratic orthogonal contrasts were used to evaluate the effect of dose. The coefficients for the unequally spaced linear and quadratic contrasts were derived using the IML procedure in SAS. In both experiments, pen was used as the experimental unit and least squares means were calculated for each independent variable. Differences were considered significant at $P \leq 0.05$ and marginally significant if $P > 0.05$ and $P \leq 0.10$.

RESULTS

Experiment 1

The AA concentrations (Table 1) of low-DON wheat were generally higher than that of the DON-contaminated wheat and differences were accounted for in diet formulation (Table 2). Nutrient analyses of experimental diets were consistent with formulated levels and increased analyzed ash content in AMMC diets correspond with the presence of additional clay (Table 3). Given the analyzed DON concentrations of the low- and high-DON wheat (Table 4), analyzed DON concentration of the PC diets accurately reflected formulated levels of <0.5 mg/kg, whereas the NC diets averaged 6.6 mg/kg DON (range 6.4 to 6.7 mg/kg), approximately 20% higher than formulated (Table 5). Although fumonisin B_1 was detected at 2.0 mg/kg in the NC diet without AMMC, analyses confirmed that no other mycotoxins were detected in PC diets above detection limits. Aflatoxin B_1 was detected at low levels (20 and 28 μg/kg) in two of three NC diets, but no other mycotoxins were in NC diets.

For pig growth performance (Table 6), a two-way DON × AMMC interaction was detected from d 4 to 7 where the addition of AMMC improved ($P < 0.05$) ADG and G:F in NC diets, but worsened ADG and G:F in PC diets. No other interactions were detected within period or overall, nor were any linear effects detected for increasing the inclusion rate of AMMC.

From d 0 to 3, pigs fed NC diets had decreased ($P < 0.001$) ADG, ADFI and feed efficiency compared to pigs fed PC diets. The addition of AMMC to diets had no effect on pig growth. A similar pattern of growth was observed from d 4 to 7, with pigs fed NC diets having decreased ($P < 0.05$) ADG, ADFI, and G:F compared to PC diets, and no differences in growth were detected for AMMC inclusion. From d 7 to 14, pigs fed NC diets continued to have decreased ($P < 0.001$) ADG driven by reduced ($P < 0.05$) feed intake, but no effect of feed efficiency was observed during this period. Once again, the addition of AMMC did not impact pig performance.

From d 14 to 21, ADG and ADFI were decreased ($P < 0.05$) for pigs fed NC diets, but feed efficiency was not affected. The addition of AMMC improved ($P < 0.05$) feed intake, but no effects on ADG or G:F were observed. During the final period (d 21 to 28), pigs fed NC diets had decreased ($P < 0.05$) ADG, but no other treatment effects were seen.

Overall (d 0 to 28), pigs fed the NC diets had reduced ADG ($P < 0.001$), driven by poorer ($P < 0.01$) ADFI and G:F, which resulted in decreased ($P < 0.001$) final BW compared to PC fed pigs. However, the addition of AMMC had no effect. Coefficient of variation of pig BW within pen tended ($P = 0.051$) to be higher in pigs fed NC diets versus those fed the PC diets.

Experiment 2

Since CP and AA levels were marginally but consistently higher in the DON-contaminated wheat (Table 1), the soybean meal fraction was increased slightly in PC diets to reflect this difference (Table 7). In the experimental diets, proximate analyses were generally in line with formulated values and the addition of AMMC was reflected by higher ash contents in those diets (Table 8). Analyzed DON concentrations (Table 9) in the naturally DON-contaminated wheat differed between NDSU (8.4 mg/kg) and LABOCEA (6.0 mg/kg). Low

levels of several other mycotoxins were detected in the DON-contaminated wheat source. To ensure that final diet DON levels were adequate to achieve a DON-associated reduction in performance, the analysis from LABOCEA was used as the basis for diet formulation. Analyzed DON in the final diets revealed levels that were within 20% of the targeted DON level, averaging 1.7 and 3.2 mg/kg for the 1.5 and 3.0 mg/kg targets, respectively (Table 10).

A DON × AMMC interaction (linear, $P < 0.05$) was observed from d 0 to 7, where increasing AMMC improved ADG in PC and low-NC diets but decreased ADG in high-NC diets (Table 11). The interaction for ADG appeared to be driven by a tendency for a G:F interaction (linear, $P < 0.05$) in which increasing AMMC inclusion worsened feed efficiency in high-NC diets whereas feed efficiency remained similar in pigs fed PC and low-NC diets regardless of AMMC inclusion. Furthermore, a tendency for a DON × AMMC interaction for feed efficiency (quadratic, $P = 0.073$) was observed from d 14 to 21, where increasing AMMC in PC and low-NC diets worsened feed efficiency, whereas in high-NC diets increasing AMMC initially improved but subsequently worsened G:F at the 0.50% inclusion rate.

For the main effects of DON and AMMC on growth performance (Table 12), from d 0 to 3, ADG, ADFI, and G:F decreased (linear, $P < 0.001$) with increasing DON concentration. From d 4 to 7, increasing DON level progressively worsened ADG ($P < 0.05$), driven not by ADFI but as a consequence of poorer ($P < 0.05$) G:F. From d 7 to 14, ADG decreased (linear, $P < 0.001$) as DON increased in the diet. This growth reduction was influenced primarily by progressively poorer (quadratic, $P < 0.001$) G:F as ADFI decreased (quadratic, $P < 0.001$) and then recovered with increasing DON concentrations. From d 14 to 21, increasing DON level tended to decrease (linear, $P = 0.087$) ADG, and increasing AMMC level tended to reduce (linear, $P = 0.094$) feed efficiency. Overall (d 0 to 21), increasing DON concentration in nursery pig diets progressively

worsened (linear, $P < 0.001$) ADG and final BW, governed predominantly by a decrease (linear, $P < 0.001$) in feed efficiency, with poorer ADFI (quadratic, $P < 0.001$) as a contributing influence. The addition of AMMC had no effect on overall ADG, ADFI, G:F or final BW.

DISCUSSION

The origin of DON used in studies appears to be important (Eriksen and Petterson, 2004; Etienne and Wache, 2008). When purified sources of DON have been used, effects on growth performance have been less severe compared to naturally-contaminated DON sources, even when no other mycotoxins were detected (Trenholm et al., 1994). While this difference is yet to be explained, proposed hypotheses include presence of other fungal components in naturally-contaminated grains that contribute to DON toxicity, differential rates or degree of DON absorption, and potential undervaluation of DON due to the difficulty of analyzing toxin in a complex grain matrix (Etienne and Wache, 2008; Pestka 2010). Finally, given that potential detoxifying agents are designed to prevent effects of naturally-contaminated feedstuffs, wheat predominately contaminated with DON was identified and used to incorporate into test diets at desired concentrations. Low concentrations of aflatoxin and fumonisin were also detected in Exp. 1 diets, but at concentrations well below safe levels, determined as less than 200 μg/kg for aflatoxin and 5 mg/kg for fumonisins (Thaler and Reese 2010). Therefore, while multi-toxin interactive effects cannot be totally excluded, in the present study, mycotoxin analyses indicate the observed growth responses were primarily due to DON.

Although rarely accounted for when testing DON-detoxifying agents, Fusarium pathogens are also known to alter the nutrient content and digestibility of the affected grain. Matthaus et al. (2004) reported higher CP and ash contents and smaller kernels in wheat inoculated with Fusarium culmorum. Thanh et al (2015) also observed increased analyzed N

concentrations in diets containing DON-contaminated wheat. However, Dänicke et al. (2004a) reported no differences in CP concentrations between wheat sources. In the present study, the high-DON wheat source was generally lower in CP and AA content in Exp. 1 and higher in AA content in Exp. 2. While Fusarium-induced fluctuations in nutrient content appear inconsistent, they highlight the need to account for differences during diet formulation so that the mycotoxin-specific effects and efficacy of detoxifying agents can be interpreted accurately. Contamination with Fusarium can also impact nutrient digestibility. In a pig growth study by Thanh et al. (2015), pigs fed DON-contaminated diets (4.6 mg/kg) had reduced DM, energy and fat digestibility. However, this conflicts with previous reports where feeding DON-contaminated diets had no impact (Dänicke et al., 2004a) or even increased total tract nutrient digestibility in feed-restricted pigs (Dänicke et al., 2004b). Authors attributed these fluctuations in digestibility to variations in grain varieties used, production of cell wall degrading enzymes by Fusarium fungi, and DON-induced changes in intestinal absorption capacity (Bracarense et al., 2012).

Unlike some other mycotoxins, DON effects on tissue composition and blood metabolites are negligible and well-characterized (Swamy et al., 2002; Madsen et al., 2013). Accordingly, these analyses were not measured in the present study. From a growth perspective, in the present studies, feeding diets containing approximately 1.7 or 3.2 mg/kg DON in Exp. 2 and 6.6 mg/kg DON in Exp. 1 decreased ADG by 10, 13 and 20%, respectively, compared to controls. A pair of meta-analyses (Dänicke et al., 2002; Etienne and Wache 2008) both calculated that once dietary DON exceeds 1 mg/kg, BW gain decreases by approximately 7% for each additional mg of DON. In the present study, pigs fed low levels of DON in Exp. 2 generally followed these predictive equations, but the effects of feeding 6.6 mg/kg DON in Exp. 1 were not as severe as projected.

While it is known that DON is more rapidly and efficiently absorbed (55%) in pigs compared to other species, and that pigs have limited ability to metabolize DON into less toxic forms (Prelusky et al., 1988; Goyarts and Dänicke, 2006; Wu et al., 2010), the variability in toxicity between individual pigs is not well characterized. During Exp. 1, within pen coefficient of variation in BW increased when pigs were fed DON-contaminated diets. This observation may indicate that some pigs may be more sensitive to DON than others, may develop a tolerance to DON more rapidly, or may have greater ability to metabolize DON. To our knowledge, the effects of DON on BW variation between similarly treated pigs has not been previously reported, but future studies should attempt to clarify this observation. Unfortunately, pens of pigs in Exp. 2 were not initially balanced for BW variation, and thus changes over time could not be evaluated.

In most pig growth studies with DON, decreased feed intake is the most commonly observed effect. This reduction in intake appears to be primarily associated with altered neuroendocrine signaling in the digestive and central nervous system of the pig, particularly via elevated levels of serotonin (Prelusky 1994; Rotter and Pestka 1996). Known to reduce intestinal motility and gastric emptying in rodents, this serotonergic effect is likely to impact pigs in a similar fashion (Fioramonti et al., 1993). However, Ossenkopp et al. (1994) also demonstrated that DON causes conditioned taste aversion in rats, mediated by the area postrema of the brain, which is likely to contribute to the anorexic effects of DON.

Previous reports indicate that unless DON levels exceed 1 mg/kg, effects on pig growth are minimal; however, each additional mg/kg DON is predicted to decrease ADFI by 4 to 5% (reviewed by Dänicke et al., 2002; Etienne and Wache 2008). In the present experiments, effects of DON on feed intake were often less severe. In Exp. 1, feeding 6.6 mg/kg DON only reduced ADFI by 10%. In Exp. 2, feeding 1.7 mg/kg DON elicited an 8% decrease in ADFI, consistent

with the prediction equation, but interestingly, ADFI was only reduced by 2% at the higher DON concentration of 3.2 mg/kg. In Exp. 1, ADFI remained suppressed throughout the study, but to a much lesser extent during the last 2 wk compared to the initial 2 wk (6% vs. 22%). In Exp. 2, lower DON levels resulted in negligible feed intake effects after the initial exposure period. These results are consistent with earlier reports where feed intake was often restored after 7 to 14 d if diets contained less than 3 mg/kg DON (Lun et al., 1985; Grosjean et al., 2002; Rempe et al., 2013). These observations also support the hypothesis that pigs develop some degree of adaptation to DON after the initial exposure period (Dersjant-Li et al., 2003). Development of tolerance to the anorectic effects of DON is congruent with observations that DON-induced taste aversion diminishes with time, which is common among anorexic compounds dependent on serotonergic mechanisms (reviewed by Rotter and Pestka, 1996).

While effects of DON on feed intake are well-characterized, DON-induced changes in feed efficiency are multidimensional and less understood. At the cellular level, DON causes cell death via apoptosis and inhibits protein synthesis by obstructing translation at the ribosomal level, leading to ribotoxic stress syndrome (reviewed by Pestka, 2010). These effects are known to have the greatest impact on rapidly dividing cells such as epithelial and immune cells in the gastrointestinal tract (GIT; Van De Walle et al., 2010). Thus, DON contamination causes compromised barrier function by decreasing the expression of tight junction proteins (Van De Walle et al., 2010; Pinton et al., 2012), and can increase the susceptibility of the GIT to bacterial infections (Grenier and Applegate, 2013). Exposure to DON also decreases the rate of epithelial cell division, resulting in flattened intestinal villi and reducing the absorptive surface area for nutrient uptake (Bracarense et al., 2012). Combined with DON-induced leukocyte apoptosis which suppresses immune function (Pestka et al., 2004), these effects are likely to contribute to

growth retardation. Conceivably, these effects indicate that toxicity of DON might be dramatically higher if exposure were to occur alongside bacterial infection. Nonetheless, the modulation of these digestive and immune functions by DON does not always affect animal growth parameters (Grenier and Applegate, 2013).

Feeding moderate levels of DON (3.5 to 6.6 mg DON) for extended periods (95 to 115 d) during the grow-finish phase consistently worsened feed efficiency in 3 experiments (Bergsjo et al., 1993; Dänicke et al.., 2004b; Patience et al., 2014). However, in short-term studies on young pigs, effects of DON on feed efficiency appear more transitory. In several growth studies, overall G:F was not affected by DON exposure (Friend et al., 1984; Pollman et al., 1985; Grosjean et al., 2002). Nonetheless, when reported by phase, pigs regularly have poorer G:F during the initial period (Pollman et al., 1985; Frobose et al., 2015). This is consistent with poorer G:F reported in experiments with shorter durations (5 to 9 d; He et al., 1993; Li et al., 2011). Similar observations were observed in both of the present experiments, with severely reduced G:F during the first 3 days of DON exposure, lessening slightly by day 7, and no longer present thereafter. This initial DON-induced feed efficiency depression still had a more marked negative effect on overall ADG than DON's impact on feed intake. In Exp. 1, it is likely that the higher DON levels fed (6.6 mg/kg) contributed to the poorer feed efficiency observed, likely mediated by previously described effects such as suppressed immune and GIT function. However, in Exp. 2, lower levels of DON were fed (1.7 and 3.2 mg/kg) and yet the effects of DON on G:F were just as severe (11%) as in Exp. 1. Health challenge may have contributed to the more marked effect of DON on feed efficiency, as pigs in Exp. 2 were concomitantly affected by influenza which originated from the source sow farm. Moreover, in both experiments, the authors observed that pigs fed DON-contaminated diets required frequent adjustment of feeders to maintain the predetermined

50% pan coverage, with DON-fed pigs being more likely to sort through the feed leading to complete feed pan coverage. This would be congruent with earlier reports that the illness-inducing effects of DON can induce conditioned taste aversion which lessens over time (Osweiler et al., 1990; Ossenkopp et al., 1994). This observation requires additional investigation, but feed wastage during this period would also contribute to poorer feed efficiency. Finally, one may question whether pigs exposed to DON in field conditions are consistently exposed to DON levels great enough to allow tolerance to develop. In large-scale commercial situations, pigs are more likely to be fed diets containing multiple sources of cereal grains and therefore may be exposed to DON intermittently, rather than continuously as has been provided in almost all experiments testing DON effects. Currently, it is unknown whether the severity of growth effects may differ when pigs are intermittently exposed to DON compared to continuous DON exposure.

Some technical treatments applied prior to feeding contaminated grains are known to partially or completely detoxify DON (e.g. physical separation, inactivation by heat/microbes, ozone or ammonia gas treatment); however, these methods have been too labor- and cost-intensive to merit widespread commercial adoption or have failed to meet government regulations (McKenzie et al., 1997; Döll and Dänicke, 2004; Young et al., 2007; Li et al., 2011). Supplementing contaminated diets with detoxifying agents is widely regarded as a more practical approach; however, currently-available feed additives have generally failed to alleviate the effects of DON (Ramos et al., 1996; Huwig et al., 2001; Awad et al., 2010). While previous attempts to use mineral adsorbing agents on non-polar mycotoxins such as DON have been ineffective (Döll and Dänicke, 2004; Döll et al., 2005), the use of AMMC had not been previously tested in vivo.

Through a patented process (Amadeite®; Olmix S. A., Brehan, France), the structure of the montmorillonite is modified using ulvans extracted from green seaweed (Lahaye and Robic, 2007). These water-soluble polysaccharides act as pillars between layers and result in a ten-fold increase of the inter-laminar space. This transformation enhanced the DON adsorptive capacity of the algae-modified montmorillonite clay (AMMC; Olmix S. A., Brehan, France) by 40% in a gastrointestinal model at low inclusion rates (0.1%; Havenaar and Demais, 2006). Nevertheless, regardless of the concentration of AMMC used and the level of DON in the diet, AMMC failed to alleviate DON-induced growth suppression in both experiments. The lack of an AMMC response in Exp. 2, when diets were fed in meal form, indicates that the heat and pressure present during pelleting was unlikely responsible for the lack of a response to AMMC in Exp. 1. Since factorial designs were used in both studies, we were able to demonstrate that AMMC supplementation also elicited no negative effects on toxin-free PC pigs. This is of note since a review by Döll and Dänicke (2003) revealed that potential detoxifying agents were actually more likely to decrease rather than improve performance in DON-contaminated diets. In many past in vivo studies, the potential detoxifying agent has only been tested in the DON-exposed group and not added to the toxin-free diet, failing to demonstrate any unspecific effects the agent may have in toxin-free control pigs. This inadequate experimental design limits interpretation of results when testing potential detoxifying agents.

In the present study, fluctuations in nutrient content in DON-contaminated versus toxin-free wheat reiterate the importance of accounting for these differences in studies assessing the impact of DON and potential detoxifying agents. Though DON contamination resulted in similar overall growth reductions to those seen in previous reports, these experiments indicate that the effects of DON on feed efficiency may be more severe than previously thought. Time-dependent

changes observed for feed intake and efficiency also appear to be important in understanding how swine producers should address future DON-contamination situations. Depending upon the growth stage and DON level in the diet, pigs appear to develop some tolerance to DON. However, the impact of intermittent, repeated exposure to DON deserves additional attention as it may actually be more often observed in field situations. Despite novel processing methods, algae-modified montmorillonite clay was ineffective at preventing the adverse effects of DON on nursery pig growth performance.

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Table 5.1. Amino acid analysis of hard red winter wheat source, Exp. 1 and 2 (as-fed basis[1]

	Experiment 1		Experiment 2	
Item, %	Low-DON[2]	High-DON	Low-DON	High-DON
Moisture	9.47	9.83	9.14	10.19
CP	12.86	10.16	11.80	12.20
AA analysis				
Lys	0.40	0.37	0.40	0.44
Ile	0.47	0.36	0.41	0.47
Leu	0.91	0.72	0.84	0.87
Met	0.21	0.16	0.21	0.22
Cys	0.28	0.22	0.27	0.27
Thr	0.37	0.30	0.36	0.38
Trp	0.18	0.12	0.15	0.17
Val	0.62	0.50	0.57	0.47

[1] Samples were analyzed for AA profile at the University of Missouri Experiment Station Chemical Laboratories in Columbia, MO.

[2] Deoxynivalenol (DON).

Table 5.2. Formulated diet composition, Exp. 1 (as-fed basis)

	Positive control (<0.5 mg/kg DON[1])		Negative control (5.0 mg/kg DON)		
Item AMMC[2]:	None	0.50%	None	0.25%	0.50%
Corn	16.90	16.40	16.33	16.20	15.88
Soybean meal, 46.5% CP	30.93	30.98	31.45	31.35	31.45
Hard red winter (HRW) wheat	46.75	46.75	---	---	---
High-DON[3] HRW wheat	---	---	46.75	46.75	46.75
Soybean oil	2.00	2.00	2.00	2.00	2.00
Monocalcium phosphate, 21% P	1.05	1.05	1.05	1.05	1.05
Limestone	1.05	1.00	1.05	1.03	1.00
Salt	0.35	0.35	0.35	0.35	0.35
Vitamin premix with phytase[4,5]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[6]	0.15	0.15	0.15	0.15	0.15
L-Lys HCl	0.33	0.33	0.33	0.33	0.33
DL-Met	0.10	0.10	0.15	0.15	0.15
L-Thr	0.14	0.14	0.14	0.14	0.14
AMMC[2]	---	0.50	---	0.25	0.50
Total	100	100	100	100	100
Calculated analysis					
SID[7] amino acids, %					
Lys	1.28	1.28	1.28	1.28	1.28
Ile:Lys	65	65	62	62	62
Leu:Lys	120	120	115	115	115
Met:Lys	31	31	33	33	33
Met & Cys:Lys	58	58	58	58	58
Thr:Lys	64	64	64	64	64
Trp:Lys	20.7	20.7	18.9	18.9	18.9
Val:Lys	72	72	69	69	69
Total Lys, %	1.42	1.42	1.42	1.42	1.42
ME, kcal/kg	3,318	3,303	3,318	3,309	3,303
SID Lys:ME, g/Mcal	3.86	3.89	3.87	3.87	3.89
CP, %	22.3	22.3	21.2	21.2	21.3
Ca, %	0.73	0.73	0.73	0.73	0.74
P, %	0.65	0.65	0.66	0.66	0.66
Available P, %	0.48	0.48	0.49	0.48	0.48

[1] Deoxynivalenol (DON).

[2] Algae-modified montmorillonite clay product (AMMC; Olmix S. A., Brehan, France).

[3] Analyzed DON concentration in HRW wheat was 10.7 mg/kg.

[4] Provided per kilogram of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D_3; 17,637 IU vitamin E; 1,764 mg vitamin K; 3,307 mg riboflavin; 11,023 mg pantothenic acid; 19,841 mg niacin; and 15.4 mg vitamin B_{12}.

[5] Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 750 phytase units phytase/kg of diet and 0.13% available P released.

[6] Provided per kilogram of premix: 22.0 g Mn from manganese oxide; 73.4 g Fe from iron sulfate; 73.4 g Zn from zinc sulfate; 11.0 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.
[7] Standardized ileal digestible.

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Table 5.3. Nutrient analysis of experimental diets, Exp. 1 (as-fed basis)[1]

		Positive control (<0.5 mg/kg DON[2])		Negative control (5.0 mg/kg DON)		
Item, %	AMMC[3]:	None	0.50%	None	0.25%	0.50%
Moisture		10.88	10.80	10.74	10.63	10.76
CP		23.00	23.26	22.29	22.04	22.51
Ether extract		3.16	3.22	2.93	3.08	3.02
Ash		5.03	5.41	5.68	5.82	5.82
AA analysis						
Lys		1.41	1.42	1.41	1.41	1.49
Ile		0.87	0.90	0.85	0.84	0.91
Leu		1.71	1.71	1.66	1.60	1.67
Met		0.43	0.41	0.45	0.41	0.49
Cys		0.38	0.36	0.35	0.32	0.36
Thr		0.90	0.90	0.84	0.86	0.89
Trp		0.30	0.29	0.28	0.30	0.30
Val		0.99	1.01	0.96	0.95	1.02

[1] Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.
[2] Deoxynivalenol (DON).
[3] Algae-modified montmorillonite clay (AMMC; Olmix S. A., Brehan, France).

Table 5.4. Mycotoxin analysis of basal ingredients, Exp. 1 (as-fed basis)

		Hard red winter wheat	
Item, mg/kg	Ground corn	Low-DON[1]	High-DON[1]
NDSU[2]			
DON	<0.50	<0.50	10.60[3]
LABOCEA[4]			
DON	---[5]	---	10.70
15-Acetyl DON	---	---	0.12
Zearalenone	---	---	0.35
Fumonisin B_1	---	---	0.03

[1] Deoxynivalenol (DON).
[2] North Dakota State University (NDSU) Veterinary Diagnostic Laboratory, Fargo, ND. Samples were sent for 18-component mycotoxin analysis and analyzed using a variety of mass spectrometry, ELISA, and HPLC methods. Included in the table are mycotoxins found at levels above detection limits (0.5 mg/kg).
[3] Mean of two duplicate samples sent to NDSU. Individual samples had DON levels of 10.0 and 11.1 mg/kg, respectively.
[4] LABOCEA, Ploufragan, France. Samples analyzed using a 43-component toxin screen using liquid-chromatography coupled with tandem mass spectrometry analysis techniques. Included in the table are mycotoxins found at levels above detection limits (10 μg/kg).
[5] (---) indicates samples were not tested.

Table 5.5. Mycotoxin analysis of experimental diets, Exp. 1 (as-fed basis)[1]

		Positive control (<0.5 mg/kg DON[2])		Negative control (5.0 mg/kg DON)		
Item	AMMC[3]:	None	0.50%	None	0.25%	0.50%
DON[2], mg/kg		<0.5	<0.5	6.6	6.7	6.4
Fumonisin B_1, mg/kg		2.0	<2.0	<2.0	<2.0	<2.0
Aflatoxin B_1, µg/kg		<20	<20	20	28	<20

[1] North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND. Samples were sent for 18-component mycotoxin analysis and analyzed using a variety of mass spectrometry, ELISA, and HPLC methods. Included in the table are mycotoxins found at levels above detection limits in at least one diet.
[2] Deoxynivalenol (DON).
[3] Algae-modified montmorillonite clay (AMMC; Olmix S. A., Brehan, France).

Table 5.6. Mycotoxin analysis of experimental diets, Exp. 1 (as-fed basis)[1,2]

	Positive control (<0.5 mg/kg DON)[2]		Negative control (5.0 mg/kg DON)[3]				Probability, P <[4,5]	
Item AMMC:[6]	None	0.50%	None	0.25%	0.50%	SEM	DON	AMMC
d 0 to 3								
ADG, g	178	233	20	24	-1	45.4	0.001	0.511
ADFI, g	410	398	302	315	293	59.0	0.001	0.677
G:F	0.396	0.558	0.038	0.075	0.012	0.109	0.001	0.369
d 4 to 7								
ADG, g	411	340	233	262	261	21.3	0.001	0.141
ADFI, g	497	485	415	436	412	85.0	0.042	0.838
G:F	0.832	0.702	0.596	0.646	0.660	0.083	0.001	0.362
d 7 to 14								
ADG, g	526	530	418	396	458	17.5	0.001	0.178
ADFI, g	704	735	558	583	611	44.6	0.001	0.161
G:F	0.747	0.718	0.743	0.686	0.751	0.032	0.571	0.690
d 14 to 21								
ADG, g	527	586	476	462	483	32.2	0.001	0.138
ADFI, g	814	858	728	734	814	37.3	0.025	0.024
G:F	0.652	0.688	0.660	0.634	0.603	0.024	0.113	0.674
d 21 to 28								
ADG, g	676	633	579	537	559	35.5	0.023	0.383
ADFI, g	1035	1022	934	907	1018	36.8	0.166	0.348
G:F	0.656	0.618	0.622	0.590	0.554	0.033	0.156	0.124
d 0 to 28								
ADG, g	533	516	420	403	421	14.2	0.001	0.581
ADFI, g	782	784	686	693	726	23.2	0.003	0.364
G:F	0.683	0.658	0.614	0.585	0.583	0.021	0.002	0.201
Pig BW, kg								
d 0	10.2	10.0	9.90	10.0	10.0	0.09	0.251	0.332
d 28	24.9	24.4	21.7	21.4	21.8	0.41	0.001	0.632
Pen CV, %								
d 0	14.1	13.8	14.2	14.4	14.7	1.00	0.226	0.763
d 28	13.6	12.4	17.1	16.4	14.8	0.015	0.051	0.249

[1] A total of 280 barrows and gilts (PIC 327 × 1050; 35 d of age) were used in this 28-d study, with 7 pigs per pen and 8 pens per treatment.

[2]Formulated levels. A high-DON wheat source was used to produce diets with 5 mg/kg DON.

[3] Analyzed DON averaged <0.5 and 6.6 mg/kg for positive and negative control diets, respectively.

[4] A two-way DON × AMMC interaction was detected (P < 0.01) from d 4 to 7 where the addition of AMMC improved ADG and G:F in negative control diets, but worsened ADG and G:F in positive control diets. No other interactions were detected within period or overall.

[5] No linear effects (P > 0.05) due to AMMC inclusion within DON contaminated diets were found. 'AMMC' contrast compares diets without AMMC to those containing AMMC at 0.50%.

[6] Algae-modified montmorillonite clay (AMMC; Olmix S.A., Brehan, France).

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Table 5.7. Formulated diet composition, Exp. 2 (as-fed basis)

	Positive control (<0.5 mg/kg DON[1])			Low negative control (1.5 mg/kg DON)			High negative control (3.0 mg/kg DON)		
Item AMMC[2]:	None	0.17%	0.50%	None	0.17%	0.50%	None	0.17%	0.50%
Corn	15.07	14.89	14.53	15.35	15.17	14.81	15.63	15.45	15.09
Soybean meal, 46.5% CP	31.58	31.60	31.62	31.25	31.26	31.29	30.92	30.93	30.96
Hard red winter (HRW) wheat	50.00	50.00	50.00	25.00	25.00	25.00	---	---	---
High-DON[3] HRW wheat	---	---	---	25.00	25.00	25.00	50.00	50.00	50.00
Monocalcium phosphate, 21% P	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Limestone	1.00	1.00	1.00	1.05	1.05	1.05	1.10	1.10	1.10
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lys HCl	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
DL-Met	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Thr	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Vitamin premix with phytase[4,5]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[6]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
AMMC	---	0.17	0.50	---	0.17	0.50	---	0.17	0.50
Total	100	100	100	100	100	100	100	100	100
Calculated analysis									
SID[7] amino acids, %									
Lys	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28
Ile:Lys	64	64	64	65	65	65	65	65	65
Leu:Lys	118	118	117	118	118	118	117	117	117
Met:Lys	31	31	31	31	31	31	31	31	31
Met & Cys:Lys	57	57	57	57	57	57	57	57	57
Thr:Lys	63	63	63	63	63	63	63	63	63
Trp:Lys	21.2	21.2	21.2	20.7	20.7	20.7	20.3	20.3	20.2
Val:Lys	68	68	68	69	69	69	70	70	70
Total Lys, %	1.43	1.43	1.43	1.43	1.43	1.43	1.43	1.43	1.43
ME, kcal/kg	3,183	3,179	3,165	3,181	3,177	3,165	3,181	3,175	3,164
SID Lys:ME, g/Mcal	4.02	4.03	4.04	4.02	4.03	4.04	4.02	4.03	4.05
CP, %	22.7	22.7	22.6	22.6	22.6	22.6	22.6	22.6	22.6
Ca, %	0.68	0.68	0.68	0.69	0.69	0.69	0.71	0.71	0.71
P, %	0.68	0.68	0.68	0.69	0.69	0.69	0.71	0.71	0.70
Available P, %	0.50	0.50	0.50	0.51	0.51	0.51	0.51	0.51	0.51

[1] Deoxynivalenol (DON).

[2] Algae-modified montmorillonite clay product (AMMC; Olmix S.A., Brehan, France).

[3] Analyzed DON concentration in HRW wheat was 6.0 mg/kg at LDA Laboratories (Ploufragan, France).

[4] Provided per kilogram of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D_3; 17,637 IU vitamin E; 1,764 mg vitamin K; 3,307 mg riboflavin; 11,023 mg pantothenic acid; 19,841 mg niacin; and 15.4 mg vitamin B_{12}.

[5] Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 750 phytase units phytase/kg and 0.13% available P released.

[6] Provided per kilogram of premix: 22.0 g Mn from manganese oxide; 73.4 g Fe from iron sulfate; 73.4 g Zn from zinc sulfate; 11.0 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[7] Standardized ileal digestible.

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Table 5.8. Nutrient analysis of experimental diets, Exp. 2 (as-fed basis)[1]

		Positive control (<0.5 mg/kg DON[2])			Low negative control (1.5 mg/kg DON)			High negative control (3.0 mg/kg DON)		
Item, %	AMMC[3]:	None	0.17%	0.50%	None	0.17%	0.50%	None	0.17%	0.50%
Moisture		9.77	9.60	9.93	10.04	9.77	9.89	9.97	9.66	9.75
CP		24.9	23.8	24.2	23.4	23.2	23.3	23.5	23.7	23.5
ADF		2.6	2.4	2.1	2.7	3.5	2.2	2.5	2.6	2.6
NDF		7.6	7.0	7.5	7.6	8.0	7.4	6.8	7.2	7.2
Ether extract		2.4	2.6	2.5	2.6	2.9	2.6	2.6	2.8	2.7
Ash		5.14	5.31	5.53	5.53	5.61	5.57	5.65	5.73	5.96
Ca		0.71	0.81	0.82	0.86	0.83	0.76	0.87	0.83	0.89
P		0.74	0.67	0.68	0.69	0.69	0.71	0.69	0.71	0.74

[1] Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.
[2] Deoxynivalenol (DON).
[3] Algae-modified montmorillonite clay (AMMC; Olmix S. A., Brehan, France).

Table 5.9. Mycotoxin analysis of basal ingredients, Exp. 2 (as-fed basis)

		Hard red winter wheat	
Item, mg/kg	Ground corn	Low-DON[1]	High-DON
NDSU[2]			
DON	<0.50	<0.50	8.40
LABOCEA[3]			
DON	---[4]	---	6.03
De-epoxy DON	---	---	0.02
15-O-Acetyl DON	---	---	0.07
3-Acetyl DON	---	---	0.03
Zearalenone	---	---	0.02
HT-2 Toxin	---	---	0.02
Ergocryptin	---	---	0.08
Ergosin	---	---	0.02
Tenuazonic acid	---	---	0.05

[1] Deoxynivalenol (DON).
[2] North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND. Samples were sent for 18-component mycotoxin analysis and analyzed using a variety of mass spectrometry, ELISA, and HPLC methods. Included in the table are mycotoxins found at levels above detection limits.
[3] LABOCEA (Ploufragan, France). Samples analyzed using a 43-component toxin screen using liquid chromatography/mass spectrometry analysis methods. Included in the table are mycotoxins found at levels above detection limits.
[4] (---) indicates samples were not tested.

Table 5.10. Mycotoxin analysis of experimental diets, Exp. 2 (as-fed basis)[1]

	Positive control (<0.5 mg/kg DON[2])			Low negative control (1.5 mg/kg DON)			High negative control (3.0 mg/kg DON)		
Item AMMC[3]:	None	0.17%	0.50%	None	0.17%	0.50%	None	0.17%	0.50%
DON, mg/kg	<0.5	<0.5	<0.5	1.7	1.8	1.7	3.4	2.7	3.5

[1] Diet samples were analyzed at North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND. An 18-component mycotoxin analysis was conducted using a variety of mass spectrometry, ELISA, and HPLC methods. Included in the table are mycotoxins found at levels above detection limits.

[2] Deoxynivalenol (DON).

[3] Algae-modified montmorillonite clay (AMMC; Olmix S. A., Brehan, France).

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Table 5.11. Interactive effects of an algae-modified montmorillonite clay (AMMC) on growth performance of nursery pigs fed diets contaminated with low levels of deoxynivalenol (DON), Exp. 2[1,2]

											Probability, $P <$	
		Positive control (<0.5 mg/kg DON)			Low negative control (1.5 mg/kg DON)			High negative control (3.0 mg/kg DON)				DON × AMMC
Item	AMMC:	None	0.17%	0.50%	None	0.17%	0.50%	None	0.17%	0.50%	SEM	Linear	Quad
d 0 to 3													
ADG, g		409	387	418	325	280	346	275	292	198	34.8	0.049	0.124
ADFI, g		629	615	631	549	539	580	519	526	531	27.3	0.862	0.691
G:F		0.650	0.630	0.657	0.590	0.513	0.597	0.524	0.555	0.370	0.042	0.019	0.069
d 4 to 7													
ADG, g		426	394	435	363	382	383	389	367	360	28.0	0.446	0.397
ADFI, g		508	502	528	540	513	490	544	540	499	34.5	0.233	0.815
G:F		0.865	0.801	0.849	0.667	0.765	0.808	0.728	0.683	0.715	0.065	0.996	0.301
d 7 to 14													
ADG, g		576	572	599	537	490	527	484	481	506	32.2	0.987	0.315
ADFI, g		832	885	924	760	722	767	908	820	882	53.7	0.258	0.843
G:F		0.70	0.646	0.651	0.710	0.689	0.700	0.547	0.596	0.583	0.031	0.272	0.722
d 14 to 21													
ADG, g		672	688	652	667	624	603	639	643	641	20.9	0.527	0.277
ADFI, g		963	955	975	931	933	920	980	919	970	84.9	0.851	0.424
G:F		0.71	0.728	0.680	0.726	0.678	0.663	0.658	0.705	0.664	0.064	0.493	0.073
d 0 to 21													
ADG, g		556	550	559	517	484	499	488	486	479	14.6	0.618	0.268
ADFI, g		785	797	824	745	726	739	807	758	788	25.7	0.300	0.884
G:F		0.71	0.691	0.681	0.696	0.669	0.678	0.609	0.643	0.608	0.025	0.559	0.177
Pig BW, kg													
d 0		11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	0.24	0.965	0.996
d 21		23.1	23.0	23.2	22.3	21.6	21.9	21.7	21.6	21.5	0.48	0.740	0.488

[1] A total of 360 barrows (PIC 1050; initially 45 d of age) were used in a 21-d experiment with 8 pens per treatment and 5 pigs per pen. All diets were fed in meal form.

[2] Algae-modified montmorillonite clay (AMMC; Olmix S. A., Brehan, France).

[3] Denotes formulated levels. High-DON wheat (6.0 mg/kg) was used to incorporate DON into diets at desired concentrations.

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Table 5.12. Main effects of deoxynivalenol (DON) and algae-modified montmorillonite clay (AMMC) on nursery pig performance, Exp. 2[1]

								Probability, *P* <			
	Formulated DON[2], mg/kg				AMMC[3], %				DON		AMMC	
Item	<0.5	1.5	3.0	SEM	None	0.17%	0.50%	SEM	Linear	Quad	Linear	Quad
d 0 to 3												
ADG, g	405	317	255	27.9	337	320	321	27.9	0.001	0.480	0.503	0.537
ADFI, g	625	556	525	23.0	565	560	581	23.0	0.001	0.144	0.222	0.411
G:F	0.646	0.567	0.483	0.028	0.588	0.566	0.541	0.028	0.001	0.938	0.144	0.817
d 4 to 7												
ADG, g	418	376	372	16.2	393	380.9	392.6	16.2	0.047	0.342	0.915	0.557
ADFI, g	513	514	528	24.9	531	518	506	24.9	0.536	0.774	0.312	0.840
G:F	0.838	0.747	0.709	0.040	0.753	0.750	0.790	0.040	0.015	0.549	0.425	0.727
d 7 to 14												
ADG, g	582	518	490	27.3	532	514	544	27.3	0.001	0.218	0.329	0.148
ADFI, g	880	750	870	41.3	833	809	858	41.3	0.764	0.001	0.352	0.293
G:F	0.665	0.700	0.575	0.018	0.652	0.644	0.645	0.018	0.001	0.001	0.809	0.800
d 14 to 21												
ADG, g	671	631	641	12.0	659	652	632	12.0	0.087	0.103	0.103	0.935
ADFI, g	965	928	956	80.6	958	936	955	80.6	0.754	0.166	0.949	0.370
G:F	0.705	0.689	0.676	0.061	0.697	0.704	0.669	0.061	0.124	0.940	0.094	0.356
d 0 to 21												
ADG, g	555	500	484	9.0	520	507	513	9.0	0.001	0.053	0.644	0.292
ADFI, g	802	737	784	17.3	779	760	784	17.3	0.357	0.001	0.629	0.233
G:F	0.694	0.681	0.620	0.021	0.672	0.668	0.656	0.021	0.001	0.039	0.206	0.889
Pig BW, kg												
d 0	11.4	11.4	11.4	0.14	11.4	11.4	11.4	0.14	0.999	0.979	0.968	0.998
d 21	23.1	21.9	21.6	0.28	22.3	22.0	22.2	0.28	0.001	0.220	0.789	0.510

[1] A total of 360 barrows (PIC 1050; initially 45 d of age) were used in a 21-d experiment with 24 replicate pens per treatment and 5 pigs per pen. All diets were fed in meal form.
[2] Denotes formulated levels. High-DON wheat (6.0 mg/kg) was used to incorporate DON into diets at desired concentrations.
[3] Algae-modified montmorillonite clay (AMMC; Olmix S. A., Brehan, France).