Effects of Nursery Floor Space Allowance on Growth, Physiology, and Immunology in Replacement Gilts

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Abstract

In U.S. swine herds, sow removal rates due to death and voluntary and involuntary culling exceed 50% annually. This loss poses an economic problem for producers because the cost of acquiring replacement females is great. Although research has shown that crowding in the nursery has negative impacts on growth, research describing effects of crowding on subsequent reproductive performance and longevity in sows is lacking. This experiment was conducted to determine the impacts of crowding during the nursery phase of production on growth, physiology, and immunology in replacement gilts. Gilts (22.3 \pm 3.2 d of age and 5.6 \pm 0.6 kg BW) were subjected to floor space allocations of 0.15, 0.19, or 0.27 m²/pig during a 7-wk nursery period. Floor space allocations were achieved by altering the number of pigs per pen (14, 11, and 8 gilts/pen, respectively). As was expected, reduced floor space allowance in the nursery negatively affected growth performance although there was inconclusive physiological and immunological evidence to suggest that pigs were experiencing highly stressful conditions. Although feed intake was not measured, changes in blood counts and blood chemistry for gilts allowed reduced floor space were similar to other studies that reported negative effects of crowding on feed consumption. Further study of the gilts involved in this study will aim to determine if there are any links between the effects of crowding during the nursery and subsequent reproductive performance and longevity in the breeding herd.

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List of Abbreviations

ACTH Adrenocorticotropic hormone
ANS Autonomic nervous system
AST Aspartate aminotransferase

AST/GOT Aspartate aminotransferase/glutamic-oxalacetic transaminase

BAND Banded neutrophils

BASO Basophils BW Body weight

CBC Complete blood count

CPK or CK Creatine phosphokinase or creatine kinase

CR Calculated requirement

CR-50 Calculated requirement less 50% of estimated free space

CRH Corticotropin-releasing hormone

HGB Hemoglobin

HPA Hypothalamic-pituitary-adrenocortical

MCH Mean corpuscular hemoglobin

HCT Hematocrit

MCV Mean corpuscular volume

MONO Monocytes

mRNA Messenger ribonucleic acid
N:L Neutrophil to lymphocyte ratio

NRBC Number of red blood cells/100 white blood cells

PP Plasma proteins

PVN Paraventricular nucleus

RDW% Red blood cell distribution width

RBC Red blood cells
RETIC# Reticulocyte number
RETIC% Reticulocyte percentage
SEG Segmented neutrophils

VMRCVM Virginia-Maryland Regional College of Veterinary Medicine

WBC White blood cells

Chapter 1. Introduction

In U.S. commercial swine herds, annual sow removal rates due to death and voluntary and involuntary culling exceeded 50% from 2005 to 2010 (Knauer and Hostetler, 2013). Voluntary culling refers to sows removed to manage the herd parity profile or females removed for farrowing difficulties, small litters, poor lactation performance or maternal behavior, or decreased productivity relative to the herd average. Typical reasons for involuntary culling include anestrus, failure to conceive, abortion, lameness, or disease. Increased sow removal rates increase the number of replacement gilts introduced into breeding herds which increases both the cost of pork production and the risk of bringing in new diseases. Replacement gilts require a minimum of three parities to pay for their replacement costs (Stalder et al., 2003; Stalder et al., 2004), and there is a need for research that increases sow longevity and lifetime reproductive performance. Sow longevity, however, is a complex trait that is influenced by many factors. Although modern swine production systems have benefited from much research focused on how management of replacement gilts during the grow-finish phase of production and the peripubertal period influences subsequent reproduction, an emerging concept is that the environment and management to which gilts are exposed prenatally or early in life has profound effects as well. For example, litter size during lactation ultimately influences both reproductive performance and sow longevity. Indeed, Flowers (2008) observed enhanced longevity, farrowing rates, and numbers of pigs born alive over three parities for sows raised in litters of seven piglets versus 10 piglets during lactation. There is a critical need to develop and evaluate best management practices for gilt development, such as the amount of floor space required during rearing, that optimizes future reproductive capacity. Floor space allowance during the nursery period can affect pig growth performance. For example, researchers from Kentucky subjected pigs to three

treatments – 6 pigs/pen and 0.50 m²/pig, 12 pigs/pen and 0.25 m²/pig, and 6 pigs/pen and 0.25 m²/pig – and observed that crowding reduced ADG in both gilts and barrows (Cho et al., 2010). The results illustrate that environmental factors in early life can impact growth. There is a lack of research, however, that describes the effects of floor space allowance during the nursery period on subsequent reproductive performance and longevity in replacement gilts.

Chapter 2. Literature Review

Sow Longevity

Introduction

The length of productive life of a sow is considered by many in the swine industry to be both an economic and animal welfare concern. As a trait, sow longevity can be defined in several ways including, but not limited to: lifetime productivity (how many pigs that sow produces while in the herd), length of life, length of herd life, and length of productive life. Average sow longevity in a herd is influenced by predetermined parities at which sows are automatically removed, sow death rate, voluntary and involuntary culling rates, replacement rate, and the percentage of gilts in the herd (Stalder et al., 2004). Inconsistencies exist across the methods used to obtain this measure making it difficult to compare sow longevity across herds or studies (Stalder et al., 2004). The suggestion has been made that mean parity at removal may be an appropriate measure for quantifying sow longevity because recording female age is not common in the swine industry (Deen and Matzat, 2003). Hoge and Bates (2011) analyzed six different definitions of longevity as they relate to herd productivity: length of productive life (days from first farrowing to removal), maximum completed parity before removal, lifetime prolificacy (number of piglets born alive during productive life), ability of a sow to achieve four parities before removal, ability of a sow to produce 40 pigs before removal, and a combination measure of length of life and productivity obtained by dividing number of piglets born alive during productive life by the difference between cull date and birth date. The six different definitions yielded similar results indicating that the optimal definition may be producer dependent (Hoge and Bates, 2011). Increasing sow longevity can be achieved by improving genetics and rearing

environment and can result in decreased replacement costs and a greater proportion of sows in a herd that have reached maximum productivity.

Annual culling rates

From 2007 to 2012, mean sow removal rates exceeded 50% for U.S. commercial swine herds that contributed data to the PigCHAMP database (PigCHAMP, 2007, 2008, 2009, 2010, 2011, 2012). The poorest 10% of farms had removal rates of 75% during the same time period. The mean removal rates include a mean culling rate of 47.2% and a mean death rate of 8.5%. High removal rates lead to a greater than ideal proportion of parity one females in the sow herd whose offspring are slower growing and have more health-related problems when compared to piglets from older parity sows (Mote et al., 2009). Logically, a sow remaining in the breeding herd for fewer parities is likely to produce fewer piglets during her productive life than a sow remaining in the herd for more parities. Thus, the lesser parity sow has reduced opportunity to be sufficiently productive (pigs weaned and sold per lifetime) to achieve a return on the replacement gilt investment cost (Stalder et al., 2004). Replacement gilts require a minimum of three parities to pay for their replacement costs (Stalder et al., 2003; Stalder et al., 2004). In the U.S. swine industry, it is most common for producers to acquire new female breeding stock by purchasing out-of-herd replacement gilts. Poor longevity results in increased replacement rate of breeding herd females which decreases profitability due to the initial cost of replacement gilts as well as costs for developing and acclimating these animals. Additionally, introducing new animals increases the risk of transmitting new diseases from the replacement animals to the existing breeding herd.

Reasons sows are culled

Voluntary culling refers to sows removed to manage the herd parity profile, but also refers to females removed for farrowing difficulties, small litters, poor lactation performance or maternal behavior, or decreased productivity relative to the herd average. Sows are involuntarily culled for anestrus, failure to conceive, abortion, lameness, or disease (Friendship et al., 1986; Stalder et al., 2004; Knauer et al., 2012). Reproductive failure is the leading overall reason females are removed from the breeding herd and includes failure to cycle, failure to farrow, and inability to conceive (Lucia Jr. et al., 2000; Stalder et al., 2004). Results from a study by Knauer et al. (2012) show that reproductive failure is the most common culling reason for parities one to five (66.1, 58.1, 52.7, 39.4, and 37.7%, respectively) and old age is the most common culling reason for parities six to 10 (30.1, 60.4, 71.0, 81.7, and 86.6%, respectively). Foot and leg injuries/lameness is also a common culling reason for younger sows (parity zero to three) (Lucia Jr. et al., 2000; Stalder et al., 2004; Anil et al., 2009). Lame sows tend to have fewer litters (< 3) and greater baby pig losses (27.7%) compared to non-lame sows (4.5 litters and 12.4% pig loss) (Anil et al., 2009). Average parity at culling ranges from three to four (Friendship et al., 1986; Stalder et al., 2004; Hoge and Bates, 2011) while there is evidence to suggest that natural longevity of sows is 12 to 15 years when performance is not a factor in removal from the breeding herd (Bazer et al., 2001). In a study by Mote et al. (2009), sows that were culled for old age averaged eight litters, however, these sows were still producing at the herd average or better. This perhaps indicates that sows should not be culled for old age before the end of their natural lifespan and in doing so, producers are foregoing profit on future unborn litters. Once sows reach a mature age (above parity three to five) culling for reproductive failure is less of an issue, and after sows achieve these parities producers can cull based largely on individual performance

(Stalder et al., 2004; Knauer et al., 2012). Because it appears that most culling occurs in lesser parity sows and the main reason for culling these females is poor reproductive performance or reproductive failure, getting younger females to exhibit a high level of reproduction seems to be the limiting factor in improving sow longevity.

There have been equivocal results concerning effects of rate of growth during rearing on reproductive performance and sow longevity. Hoge and Bates (2011) found that gilts that grew slower and were fatter had a decreased risk of being culled. Similarly, Knauer et al. (2010) found that increased ADG was associated with decreased sow retention to four parities. On the other hand, Stalder et al. (2005) found no relationship between ADG and longevity in U.S. Landrace populations. Increased backfat thickness has been shown to be positively associated with increased longevity (Stalder et al., 2005; Knauer et al., 2010). However, Serenius and Stalder (2007) found that backfat thickness at 100 kg had no effect (P = 0.40) on the risk of a sow being culled. Knauer et al. (2010) concluded that ADG and gilt backfat thickness had less effect on sow retention to parity four than age at first farrowing and age at puberty.

Economic impact of poor longevity

Early removal of sows from the herd results in negative effects on mean litter size, number of litters per sow per year, and number of pigs weaned per sow per year, thus increasing the cost per weaned pig (Anil et al., 2009). Assuming a net income of \$50 per parity, 2.0 and 3.68 parities are required to recover the initial investment (replacement) cost of the gilt under zero and 10% discount rates (a combination of inflation, interest rates, and risk), respectively. On the other hand, 10 parities are required to recover the investment cost if the net income is \$10 per litter and the discount rate is zero (Rodriguez-Zas et al., 2003). More net income will be generated from sows remaining in the herd for a longer period (Rodriguez-Zas et al., 2003).

Stalder et al. (2003) reported that a sow must remain in the breeding herd until parity three to reach a positive net present value. When culled at an average parity of three to four, sows are just becoming profitable, suggesting that producers are missing out on profits by not retaining sows for more parities. According to Anil et al. (2009), financial loss associated with lameness is estimated to be \$52/sow due to fewer pigs born, increased baby pig mortality rates, and earlier slaughter. Sows reach peak productivity during parities three to six and sows culled after the first or second parity are unprofitable (Stalder et al., 2003; Flowers, 2008). When sows are left in the breeding herd for more parities, gilt development costs are spread over more pigs produced which lowers the costs. Sow removal may not depend on biological factors of the sow alone, but may also be influenced by the number of gilts available for breeding and the market for culled sows (Anil et al., 2009).

Developmental Impacts on Growth and Reproductive Performance

Prenatal environment

The environment and management to which gilts are exposed prenatally or early in life has been shown to impact reproduction in adulthood. Oogenesis (transformation of oogonia to oocytes) in the pig begins at d 40 of fetal life (Black and Erickson, 1968) and lasts until at least d 35 postpartum (Fulka et al., 1972). Only about 50% of the cells survive transformation due to degeneration of the germ cell population (Black and Erickson, 1968). Oocytes that remain after transformation enter a state of meiotic arrest known as the dictyotene phase. These cells form the pool from which developing oocytes emerge (Rutledge, 1980). Da Silva-Buttkus et al. (2003) reported that at birth, small pigs (mean BW of 0.7 kg), often seen in large litters, had more primordial follicles, but fewer primary and secondary follicles than heavier birth weight

littermates (mean BW of 1.5 kg) indicating that intra-uterine growth retardation delayed follicular development.

Estienne and Harper (2010) conducted an experiment during which gilts were housed during gestation in individual crates, group pens throughout gestation, or crates for the first 30 d post-mating and group pens for the remainder of gestation. Gilt offspring showed similar growth performance in the nursery. Gilts farrowed by females housed in crates throughout gestation were heavier during the last 4 wk of grow-finish (P = 0.04) and tended (P < 0.09) to have less backfat than gilts farrowed by females housed in group pens throughout gestation. Mean age at puberty was not affected by treatment (P = 0.61), but fewer gilts farrowed by females housed in crates (P = 0.03) reached puberty by 165 d of age compared to the other treatment groups. The researchers suggested that gestation housing may affect future growth and reproductive performance in gilt offspring by fetal programming (Estienne and Harper, 2010). However, Kattesh et al. (1979) subjected gestating sows to elevated temperature and crowding to examine the effects of prenatal stress on reproductive behavior and physiology of male offspring. There were no significant impacts of prenatal stress on plasma testosterone concentrations or libido in boars. In swine, the neural pathways that affect reproductive behavior are still being modified as late as three mo of age which suggests that some aspects of reproductive behavior in pigs may not be impacted by prenatal stress (Lay, 2000). In another study, sows were subjected to injections of adrenocorticotropic hormone (ACTH) weekly throughout the second half of gestation to examine if prenatal stress affects the future function of the hypothalamic-pituitaryadrenocortical (**HPA**) axis in swine (Lay, 2000). Piglets from litters born to stressed sows (n = 9) and piglets from litters born to control sows (n = 9) were killed at 12 h, 1 mo, and 2 mo of age and the hypothalamus, pituitary gland, and adrenal gland were collected. Prenatally stressed pigs

had lesser birth weights, lesser anogenital distances (in females only), greater corticotropinreleasing hormone (CRH) and less β -endorphin in the hypothalamus at one mo of age, greater mRNA for the adrenal ACTH receptor at one mo of age, and larger pituitary glands at two mo of age compared to pigs farrowed by control sows. Researchers suggested that the prenatally stressed pigs would have a vigorous response to a stressor when challenged later in life because a greater number of CRH and ACTH receptors would cause a heightened increase in plasma glucocorticoids (Lay, 2000).

Neonatal environment

Current production systems require very young livestock to adjust to elevated social pressures caused by high stocking densities, physical and mental stress due to a lack of ability to carry out a range of normal behaviors, and the close proximity to humans, all of which can negatively impact growth and reproduction (Lay, 2000).

Nelson and Robison (1976) reported that at d 25 post-mating, gilts that had been raised in litters of 6 pigs prior to weaning had more corpora lutea (an indication of ovulation rate) and embryos than did gilts raised in litters of 12 pigs. Flowers (2008) conducted a 2×2 factorial study in which gilts were reared in litters of ≤ 7 pigs or ≥ 10 pigs during lactation and provided boar exposure for puberty stimulation at 140 or 170 d of age. Gilts were sent to commercial sow farms between 190 and 210 d of age. Being raised in small litters had a positive effect (P < 0.05) on sow longevity as 56.3% of gilts from small litters remained in the herd after three parities, compared to only 37.6% of gilts raised in large litters. Earlier boar exposure had a positive effect on longevity as 50.9% of gilts exposed to a boar beginning at 140 d of age remained in the herd after three parities compared to only 39.3% of gilts exposed to a boar beginning at 170 d of age (P < 0.05). Approximately 27% more gilts raised in small litters and exposed to boars at 140 d of

age were still in production after weaning three litters compared to gilts raised in large litters and exposed to boars at 170 d of age (60% vs. 33%, respectively). Being raised in a small litter increased farrowing rate by 4% (P < 0.05) and earlier puberty stimulation resulted in a 6% improvement in farrowing rate (P < 0.05). Sows raised in litters of ≤ 7 pigs and given boar exposure at 140 d of age had a 10% greater farrowing rate compared to gilts raised in litters of \geq 10 pigs and given boar exposure at 170 d. There were effects of litter size \times age at puberty stimulation for numbers of pigs born alive (P < 0.05). Gilts raised in small litters and exposed to a boar beginning at 140 d of age had 0.6 greater (P < 0.05) pigs born alive compared with gilts raised in large litters and exposed to a boar beginning at 140 d. Gilts raised in smaller litters had 0.5 more pigs per litter (P < 0.05) through three parities than gilts raised in large litters. Gilts raised in small litters had 0.9 greater pigs per litter (P < 0.05) in parity three when compared to gilts raised in large litters (11.5 vs. 10.6, respectively). Rutledge (1980) also found that replacement gilts raised in small litters (mean of 5.8 pigs/litter) had one more pig per litter (P < 0.05) than gilts raised in large litters (mean of 10.1 pigs/litter). Conversely, Serenius and Stalder (2007) found that the effect of litter size in which the sow was born was not significantly associated with length of productive life. The study by Flowers (2008) suggests that limiting litter size of future replacement gilts to seven pigs or less and providing boar exposure at 140 d, rather than 170 d of age, would increase sow longevity and each give an improvement of one pig per litter, and when implemented simultaneously would give an expected improvement of six total pigs through three parities.

The mechanisms by which the neonatal environment of gilts affects future reproduction have not been fully elucidated. Kirkpatrick and Rutledge (1988) found that there was no difference (P > 0.15) in ovulation rate between gilts reared in small litters (five piglets) or large

litters (10 piglets). However, gilts reared in small litters tended (P < 0.10) to have more embryos at 30 d of gestation than gilts reared in large litters. Hoge and Bates (2011) reported that decreased age at first farrowing improved reproductive performance and longevity (P < 0.001). This coincides with research by Serenius and Stalder (2007) which found that the greater age at first farrowing, the greater the risk of a sow being culled (P < 0.001). Knauer et al. (2010) also observed that an older age at puberty or age at first farrowing indicates sow reproductive problems later in life. However, there is concern over breeding immature gilts and it has been recommended that the optimal age at first conception is 200 to 210 d of age (Serenius and Stalder, 2007).

Nursery environment

Modern hog facilities are expensive to construct and operate. Producers must maximize pig ADG in a given facility within a specific time period to minimize the facility cost per unit of pork produced (Kornegay and Notter, 1984). In order to achieve maximum growth, it is important to know the relationship between floor space allowance and pig performance. An adequate floor space allowance gives pigs sufficient space for drinking water, lying, and feeding. The main requirements for a pig during feeding are the ability to get to, and remain at, the feed trough without undue competition from pen mates (Cho and Kim, 2011). Adequate space allowance is important for a stable dominance hierarchy. For pigs housed in space restricted environments, the hierarchy is less stable due to the inability of subordinate pigs to retreat from a threat or act of aggression displayed by a dominant pig (Cho and Kim, 2011). The National Pork Board recommends that pigs between 5.4 and 13.6 kg BW be housed at 0.15 to 0.23 m²/pig, pigs between 13.6 and 27.2 kg BW at 0.27 to 0.37 m²/pig, and pigs 27.2 to 45.6 kg BW at 0.46 m²/pig (National Pork Board, 2003). The impact of post-weaning stressors, including floor space

allowance and group size during nursery and grow-finish stages, on subsequent reproductive performance has not been adequately studied.

Nursery group size

Wolter et al. (2000) studied the effects of group size (20 [Small] or 100 [Large] pigs/pen) and floor-space allowance (calculated requirement [CR] or calculated requirement less 50% of estimated free space [CR-50]) on growth performance using 1,920 crossbred weanling pigs (5.3 ± 0.7 kg BW). From wk one through four after weaning, small and large groups at CR were both allowed 0.17 m²/pig and small and large groups at CR-50 were allowed 0.15 m²/pig and 0.13 m²/pig, respectively. From wk five through nine after weaning, all CR pigs were allowed 0.38 m²/pig and for CR-50 pigs, small and large pigs were allowed 0.32 m²/pig and 0.28 m²/pig, respectively. Piglets in large groups weighed 2, 4, and 5 % less (P < 0.001) at the end of wk 1, 4, and 9, respectively, and had decreased ADG (6%, P < 0.001) throughout the trial compared to piglets in small groups. Compared to CR-50 pigs, piglets reared at CR had greater ADG (5%, P < 0.01) throughout the trial, greater G:F (P < 0.05) for wk one through four, and greater BW (P < 0.05) (0.01) at the end of wk 4 (3%) and 9 (4%). Pigs reared in large groups also had greater (P < 0.05) variation in BW at the end of wk 9 compared to pigs in small groups. Gehlbach et al. (1966) found that pigs reared in groups of 16 gained BW slower (P < 0.01) than pigs reared in groups of 12 or 8 while floor space/pig was held constant. Kornegay and Notter (1984) suggested that increasing group size while holding floor space per pig constant reduced pig performance very little and concluded that feeder space allowance had little effect on performance. Gain to feed ratios tended (P < 0.09) to increase as number of weanling pigs per pen increased indicating improvement in feed efficiency in larger groups.

Nursery floor space

A review of the literature shows that reducing floor space allowances during the nursery period negatively affects pig growth. Wolter et al. (2000) found that, compared to CR-50 pigs, pigs reared at CR had greater ADG (5%, P < 0.01) throughout the trial, greater G:F (P < 0.05) for wk one through four, and greater BW (P < 0.01) at the end of wk 4 (3%) and 9 (4%). Variation in BW within a pen was not affected by floor-space allowance at any time during the study (P > 0.05). Gehlbach et al. (1966) found that growth to 50 kg was less (P < 0.01) for pigs reared at 0.18 m²/pig compared to 0.36 and 0.54 m²/pig. Wolter et al. (2002) found that pigs housed at double stocking density (104 pigs/pen and 0.325 m²/pig) had less (P < 0.001) ADG and BW through 10 wk post weaning compared to pigs housed at single density (52 pigs/pen and 0.650 m²/pig). Pigs housed at double stocking density also had less (P < 0.001) ADFI, but similar G:F (P > 0.10) during the first 10 wk after weaning compared to pigs housed at single density. Brumm et al. (2001) imposed floor space allocations of 0.16 m²/pig and 0.25 m²/pig during a five wk nursery phase by housing pigs in groups of 18 and 12, respectively. Crowding decreased ADFI (0.609 vs. 0.683 kg/d; P < 0.001) and ADG (0.364 vs. 0.408; P < 0.001).

Lindemann et al. (1987) conducted four studies to determine effects of feeder space on weaned pig performance and to discern if the relationship was influenced by stocking density. In the first experiment, 180 weaned pigs (28 ± 4 d of age and 6.7 kg BW) were housed in groups of 5 or 10 (floor spaces of $0.22 \text{ m}^2/\text{pig}$ and $0.11 \text{ m}^2/\text{pig}$, respectively) and given either three or six 15.2 cm sections of feeder space. There were no significant effects of stocking density × feeder space during the 4 wk study. Pigs housed in groups of 10 had reduced ADFI (P < 0.01) and ADG (P < 0.01) compared to pigs housed in groups of 5. There were no effects of reduced feeder space on any growth trait measured. The second experiment used 216 pigs (27 ± 4 d, 6.7

kg) housed at $0.12 \text{ m}^2/\text{pig}$ and provided three, six, or 12 feeder spaces during a 6 wk nursery phase. The third experiment used 240 pigs ($28 \pm 6 \text{ d}$ of age and 7.5 kg) housed at $0.15 \text{ m}^2/\text{pig}$ and provided two, four, or six feeder spaces. Growth performance was not affected (P < 0.05) by treatment in experiments 2 or 3. The fourth experiment used 180 pigs ($28 \pm 4 \text{ d}$ of age and 8.0 kg) housed in groups of 5 or 10 pigs/pen and allowed two, four, or six sections of feeder space per pen. Reductions in feeder space allowance decreased (P < 0.01) ADFI and ADG. Negative effects on ADFI and ADG were observed in pigs allowed two feeder spaces and there were no differences between groups allowed four or six spaces. Increased stocking density reduced ADFI (P < 0.05) and ADG (P < 0.01). There were no effects of stocking density × feeder space allowance (P > 0.05) in the fourth experiment. It is important to note that group size and floor space allowance were confounded in these experiments, as the different floor space allowances were achieved by changing the group size.

In a review of the literature, Kornegay and Notter (1984) concluded that ADG and ADFI increased as floor space per pig increased, but the effect on feed conversion efficiency was much smaller. Thus, it was concluded that increased ADG was primarily due to increased ADFI, which agrees with the findings of Lindemann et al. (1987) summarized in the preceding paragraph. Efficiency of floor space decreases in a quadratic manner as floor space per pig increases (Kornegay and Notter, 1984). Weanling pigs showed decreased ADG per m² by 32.6% and decreased G:F per m² by 38.9% for each 0.1 m² change in floor space per pig from 0.18 m²/pig. Floor space allocation has a great effect on pen efficiency and the effect lessens as pigs become larger. Increasing floor space allocation to maximize individual pig performance is inconsistent with maximizing the amount of pork produced in a given amount of floor area.

Kim et al. (2008) utilized 132 weanling pigs allotted to four treatments in order to study the effects of crowding stress during the nursery period on growth and subsequent reproductive performance for two parities. The following treatments were imposed over a 6 wk nursery phase: 1) 6 pigs/pen at $0.50 \text{ m}^2/\text{pig}$, 2) 9 pigs/pen at $0.33 \text{ m}^2/\text{pig}$, 3) 12 pigs/pen at $0.25 \text{ m}^2/\text{pig}$, and 4) 6 pigs/pen at $0.25 \text{ m}^2/\text{pig}$. As the number of pigs/pen increased, ADFI and ADG decreased linearly (P < 0.01 and P = 0.04, respectively). When holding constant the number of pigs/pen, pigs with restricted floor space (Treatment 4) tended (P = 0.09) to have lower ADG than pigs housed with adequate floor space (Treatment 1). With constant floor space allowance, pigs reared with a great number of pigs/pen (Treatment 3) had less ADFI (P = 0.02) than pigs with a lower number of pigs/pen (Treatment 4). Total pigs born live in parity one was unaffected by treatments (P = 0.60). However, in parity two, total pigs born live (linear for Treatments 1 to 3, P = 0.24) and live pigs born/litter (quadratic for Treatments 1 to 3, P = 0.30) were numerically decreased by crowding stress.

Grow-finish environment

A study by Young et al. (2008) utilized 1,256 replacement gilts (38 kg BW and 75 d of age) to determine the effect of floor space allowance during rearing and age at puberty on total pigs produced and removal rate over three parities. Gilts were allotted to one of two treatments: Treatment 1: gilts were given a space allowance of 1.13 m²/gilt (15 gilts per pen) and Treatment 2: gilts were given 0.77 m²/gilt (22 gilts per pen). Thus, the number of pigs/pen and floor space allowance were confounded. In addition to being weighed at the beginning and end of the experiment, gilts were ultrasonically scanned for backfat thickness and loin muscle depth, and feet and legs scored for structure, movement, and toe evenness before leaving the rearing site. Gilts were exposed to a vasectomized boar beginning at 140 d of age and age at puberty was

recorded for gilts reaching puberty before leaving the rearing site (at approximately 160 d of age). Gilts were then moved to a gilt breeding farm and when confirmed pregnant were moved to one of nine sow farms where they remained until being removed from the herd. Space allowance during rearing had no effect (P < 0.29) on growth rate in rearing, backfat thickness, loin depth, total pigs produced, or removal rate. A greater percentage of gilts given the greater space allowance in rearing attained puberty (P = 0.02) and attained puberty at a younger age (P <0.01). Gilts given the lesser floor space allowance had more (P = 0.04) cracks on their rear hooves. Gilts attaining puberty at < 185 d of age had a greater growth rate in rearing, greater backfat thickness at 200 d of age, and produced more (P < 0.05) pigs over parities one to three. Gilts gaining > 860 g/d had greater (P < 0.05) total born in parity one, but total pigs produced to the end of parity three was not different (P = 0.47) between treatments. Greater growth rate did not negatively affect removal rate. Gilts first serviced between 240 to 260 d of age produced more (P < 0.01) pigs by the end of parity three than those first serviced at > 265 d of age. A greater (P < 0.01) percentage of gilts first serviced at > 280 d of age were removed by the end of parity three.

Kuhlers et al. (1985) conducted a study with 109 Duroc × Landrace crossbred gilts that were reared in equal size pens with eight or 16 pigs/pen. Gilts in pens of eight were allowed 1.06 m^2/pig from 30 to 65 kg BW and 1.25 m^2/pig from 65 to 100 kg BW. Gils in pens of 16 were allowed half as much space per animal during each phase of production. Sows raised in pens of eight farrowed one more pig/litter (P < 0.05) and 0.7 more live pigs/litter (P = 0.15) than sows in groups of 16. There were no differences between treatments in the number of corpora lutea at 30 d of gestation (P = 0.33) or in litter birth weight (P = 0.26). Interestingly, when data were adjusted by covariance for number of live pigs at the beginning of lactation, sows from the

groups of 16 pigs/pen had greater (P < 0.01) litter size weaned and 3.1 kg greater (P < 0.05) litter weight at 21 d. Moreover, litters raised by sows that were reared in groups of 16 had 7% greater (P < 0.02) survival rates compared to those of sows reared in groups of eight.

Quantifying Stress in Swine

Stress is a general term used to describe environmental factors eliciting adaptive mechanisms by the body in an attempt to restore homeostasis (Mormede et al., 2007). Measuring activity of the HPA axis activity is the classic approach used to study stress and welfare in farm animals. In swine, CRH is synthesized in the paraventricular nucleus of the hypothalamus (PVN) and is released from the median eminence, traveling via the hypothalamo-hypophyseal portal vasculature to the anterior pituitary gland. There, CRH stimulates adrenocorticotrophs to secrete adrenocorticotropic hormone (ACTH) into the circulation which travels to the adrenal cortex where it stimulates secretion of cortisol. Cortisol is a cholesterol-derived steroid that is synthesized in the fascicular zone of the adrenal cortex under the control of ACTH. The PVN also receives signals from other hypothalamic nuclei, the brain stem, the subfornical organ, and the limbic system. These multiple inputs allow the HPA axis to respond to both internal and external stimuli. Cortisol exhibits negative feedback on the HPA axis by acting on the pituitary corticotrophs, the PVN, and higher levels in the central nervous system. This feedback is what allows the HPA axis to return to basal levels after stimulation (Mormede et al., 2007). The HPA axis, along with the autonomic nervous system (ANS), is responsible for metabolic homeostasis and regulating energy fluxes (Dallman et al., 1993). Thus, in response to stress they are able to produce energetic metabolites either from energy storing tissues (ANS mobilizes fat from adipose tissues and glycogen from liver) or by transformation of proteins into energetic metabolites (neoglucogenesis enhanced by glucocorticoid hormones) to provide energy to cope

with the stressor (Mormede et al., 2007). These systems can also be activated in situations that are not normally considered stressful, such as increased cortisol levels after a meal. The biological response to acute stressors (parturition, castration, weaning, mixing of animals, restraint, transportation, slaughter) activates the HPA axis to ultimately increase circulating cortisol levels. If the challenge is prolonged, then circulating levels of corticosteroids return to baseline even though the animal is still enduring a stressful situation (Mormede et al., 2007). Physical and social environments of farm animals including nursery pigs, such as group size and floor space allowance, are prolonged stressors that affect the HPA axis. There is great variability across species, breeds, and individuals in the response and adaptation of biological mechanisms in dealing with stress (Mormede et al., 2007).

The main technique used to assess adrenal function in pigs is to collect blood by direct venipuncture from the jugular vein and then quantify cortisol in harvested serum or plasma.

Alternatively, corticosteroids can be measured in saliva, urine, milk, or feces (Mormede et al., 2007). One problem limiting circulating levels of cortisol as a measure of a stress response is the inherently large variation in concentrations among animals. Cortisol release is subject to diurnal rhythms with levels increasing just after midnight and peaking in the morning before declining to an afternoon trough. Also, catching and restraining an animal for blood collection in itself can cause the cortisol concentrations to increase; researchers can avoid this by obtaining blood samples within two to three minutes of initially catching the animal (Mormede et al., 2007).

When taking multiple samples over an extended time period, a catheter can be surgically placed in a vessel to make sampling easier on the animal and the handler (Mormede et al., 2007).

Indeed, a thorough assessment of the effects of a chronic stressor on circulating cortisol levels necessitates a time series of cortisol measurements. Marco-Ramell et al. (2011) used eight Duroc

 \times (Landrace \times Large White) male pigs (18 to 20 kg BW) housed in a pen with a floor space allowance of 0.50 m²/pig (low density) at d 1that was changed to 0.25 m²/pig (high density) from d 15 to d 18 and d 22 to d 25. The high density was achieved by moving the fence. Blood sample analysis revealed that short-term high density housing did not alter (P = 0.456) serum cortisol concentrations.

Kornegay et al. (1993b) utilized 96 weanling pigs (8.4 kg BW and 26 d of age) to determine the effects of floor space allowance and dietary selenium and zinc levels on growth performance, clinical pathology measurements, serum mineral concentrations, and enzyme activity measured weekly, and on plasma corticosteroids, liver enzymes, and adrenal weights measured at the end of the 6-wk trial. Pigs were housed in groups of four at either 0.28 m²/pig or $0.14 \text{ m}^2/\text{pig}$. The number of lymphocytes was less (P < 0.02) for pigs housed with restricted floor space than for those housed with adequate floor space (7.43 vs. $8.02 \pm 0.28 \times 10^3 / \mu L$). The space by week interaction was significant (P < 0.05) for hematocrit, hemoglobin, lymphocytes, and total leukocytes. At the end of the 6-wk experiment, pigs housed with restricted floor space had greater (P < 0.001) hematocrit and hemoglobin concentrations than pigs housed with adequate floor space (39.02 vs. 36.54% and 12.39 vs. 11.54 g/dL, respectively). Values for total leukocytes and lymphocytes were greater (P < 0.001) for pigs housed with adequate space than for those housed with restricted space $(14,504 \text{ vs. } 13,108 \times 10^3/\mu\text{L} \text{ and } 9,249 \text{ vs. } 7,486 \times 10^3/\mu\text{L},$ respectively). Banded, segmented, and total neutrophils, monocytes, eosinophils, and basophils were not affected by floor space or floor space × wk. The total neutrophil:lymphocyte ratio (N:L), elevated ratios of which are an indication of stress (Gross and Siegel, 1983), was not influenced by floor space allowance or the interaction of floor space and time. Serum glucose was greater (P < 0.04) throughout the study for pigs housed with adequate space compared to

those housed with restricted space. At the end of the trial, urea N, an indication of dietary intake of protein and patterns of utilization of amino acids (Whang and Easter, 2000), was greater (floor space \times wk, P < 0.01) for pigs housed with adequate space compared to those with restricted space. Serum concentrations of protein, albumin, and globulin levels were not affected by floor space allowance or floor space \times wk. Serum concentrations of Ca were greater (P < 0.02) for pigs housed with adequate space than for those housed with restricted space for all weeks, except wk 4. Serum P concentrations were greater (P < 0.008) for pigs housed with adequate space than for those housed with restricted space. Serum Na and Cl concentrations were not affected by space allowance or space allowance \times wk. There was an effect of space \times week (P < 0.001) for serum K concentrations and, relative to pigs housed with adequate floor space, levels were greater during wk 1 and 2 and less during wk 3 through 6 for pigs housed with restricted space. Serum Mg was not affected by space allowance. Serum aspartate aminotransferase (AST), an indication of liver damage (Yu et al., 2013), was not influenced by space allowance. Serum creatine phosphokinase (**CPK**) was greater (space allowance \times wk, P < 0.04) for pigs housed with adequate space during wk 3, 4, and 5, but not during other weeks. The researchers concluded that the lower number of circulating lymphocytes indicated the pigs housed with restricted floor space were displaying a stress response even though corticosteroid levels were unchanged. Corticosteroid measured 38.7 and 36.3 ng/mL at the end of the trial for gilts housed at 0.28 and 0.14 m²/pig, respectively. Lymphocyte levels are highly sensitive to corticosteroid levels and increased cortisol decreases lymphocyte levels (Kornegay et al., 1993b). Gross and Siegel (1983) suggested that the heterophil:lymphocyte ratio was a sensitive measure of stress in chicks. Thus, heterophil (in the case of the pig, neutrophil) to lymphocyte ratio may be a better measure of long-term changes in the environment, and changes in corticosteroid concentrations,

a better measure of short-term changes (Gross and Siegel, 1983). An increasing ratio of heterophils (neutrophils):lymphocytes is indicative of stress because heterophils (neutrophils) increase and lymphocytes decrease due to the stressor. Kornegay et al. (1993b) did not observe an increase in N:L in response to decreased floor space although lymphocytes decreased by 13% from wk 3 to 6. Yen and Pond (1987) reported increased N:L at d 14 and 28 for pigs (7.5 kg BW) housed in groups of 16 (0.13 m²/pig) compared to groups of 8 (0.26 m²/pig). A subsequent study using the same housing conditions produced no such effect. The N:L ratios reported by Kornegay et al. (1993b) were twice as high as Yen and Pond (1987) while the concentrations of leukocytes and lymphocytes were twofold greater in Yen and Pond (1987). Lower levels of Ca and P in pigs housed with restricted floor space were seen as indicators of subtle stress (Kornegay et al., 1993b). Corticosteroids decrease renal tubular absorption of Ca which, in turn, lowers serum Ca concentrations (Kornegay et al., 1993b). Persistently low serum Ca concentrations would stimulate the parathyroid gland to increase secretion of parathyroid hormone, which would increase excretion of P through the urine effectively lowering serum P (Kornegay et al., 1993b). Additionally, reduced Ca, P, and K in pigs housed with restricted floor space may have been from reduced feed intake. In agreement with Kornegay et al. (1993b) and Yen and Pond (1987), Kornegay et al. (1993a) reported no effects of restricting floor space allowance on adrenal weight although growth performance was decreased. Kornegay et al. (1993a) reported no effect of restricting floor space on cortisol concentrations after 6 wk. However, Lindemann et al. (1987) reported greater (P < 0.01) corticosteroid activity for pigs (8.0 kg BW) housed in groups of 10 compared to groups of 5. In the Lindemann et al. (1987) study, greater serum K and CPK values for pigs with adequate space relative to pigs housed with restricted space suggests pigs with adequate space suffered more traumatic musculoskeletal

damage. Creatine phosphokinase and K are leaked into the serum proportional to the degree of damage that occurs to muscle cells (Kornegay et al., 1993b). Kornegay et al. (1993b) noticed that pigs given less space moved around less and fought less frequently than pigs given more space. Necropsy showed that adequately housed pigs suffered more traumatic injuries as evidenced by bruises and lameness. From this study researchers concluded that restricting floor space was not significantly stressful and only reduced ADG by reducing ADFI. Although there were significant differences in some blood parameters, all values were generally within limits considered normal in pigs (Kornegay et al., 1993b).

Puppe et al. (1997) found that N:L increased one day after weaning, but declined to preweaning levels by d 4 post weaning. Moreover, blood glucose concentrations of pigs relocated at weaning to unfamiliar environments were increased compared to animals weaned but kept in a familiar environment.

Conclusion

Poor sow longevity is a persistent problem in the U.S. swine industry that increases the cost of production. Events experienced early in life have been shown to negatively impact reproductive performance and longevity in sows later in life. However, the effect of floor space allowance during the nursery phase of production on sow reproductive performance and longevity has not been adequately studied. If poor reproductive performance is in fact caused by social stress during the nursery phase of production and not a result of genetic potential of a sow then the cost of replacement gilts can be reduced if producers can reduce this stressor in early life. In this thesis, the effect of nursery floor space allowance on growth, physiology, and immunology in replacement gilts was examined. Further study will determine the effects of floor space allowance in the nursery on subsequent sow performance and longevity.

Chapter 3. Effects of Nursery Floor Space Allowance on Growth, Physiology, and Immunology in Replacement Gilts

Introduction

In U.S. commercial swine herds, sow removal rates due to death and voluntary and involuntary culling exceeded 50% annually from 2005 to 2010 (Knauer and Hostetler, 2013). For producers to remain competitive there is a need for strategies to increase sow lifetime productivity and longevity. Sow longevity, however, is a complex trait that is influenced by many factors. Modern swine production systems have benefited from much research focused on how management of replacement gilts during the grow-finish phase of production and the peripubertal period influences subsequent reproduction. However, an emerging concept is that the environment and management to which gilts are exposed prenatally or early in life has profound effects as well. For example, Flowers (2008) observed enhanced longevity, farrowing rates, and numbers of pigs born alive over three parities for sows raised in litters of seven piglets versus 10 piglets during lactation. Additionally, crowding in the nursery phase of production has been shown to negatively impact pig growth performance (Kornegay and Notter, 1984; Lindemann et al., 1987; Brumm et al., 2001; Wolter et al., 2002). There is a lack of research, however, that describes the effects of floor space allowance during the nursery period on subsequent reproductive performance and longevity in replacement gilts. Work in our laboratory is focused on the development and evaluation of best management practices for gilt development, such as the amount of floor space required during rearing, that optimizes future reproductive capacity. The objective of this experiment was to determine if floor space allowance during the nursery phase of rearing in a commercial setting affects growth, physiology, and immunology in future replacement gilts.

Materials and Methods

The experiment was conducted at a multiplication farm located in Cameron, NC that was owned and operated by Murphy-Brown LLC (Rose Hill, NC). The Institutional Animal Care and Use Committee of Virginia Polytechnic Institute and State University (Blacksburg, VA) approved the experimental protocol.

Animals, Housing, and Experimental Design

A total of 2,537 maternal line gilts (22.3 ± 3.2 d of age and 5.6 ± 0.6 kg BW) (Smithfield Premium Genetics, Rose Hill, NC) from 13 consecutive groups of weaned pigs (n = 194.2 ± 8.3 gilts/group) were utilized. All animals were individually identified with numbered ear tags at birth. After weaning, gilts were housed in an enclosed nursery barn that was divided into seven rooms that utilized negative pressure mechanical ventilation and hanging gas furnaces for supplemental heating. Pen floors were woven wire. Each room contained 40 pens, 20 pens designated for gilts and 20 pens for barrows, that each measured 1.52×1.52 m. A single nipple waterer was located in each pen. One fence line feeder served two pens. Each pen had four, 15.2 cm wide feeder spaces. Pigs were allowed ad libitum access to water and feed formulated by Murphy-Brown, LLC to meet or exceed the requirements for the various nutrients (NRC, 1998). Three diets were sequentially fed in a commercial phase feeding program. A single room was loaded each week with weaned barrows and gilts.

A 3×3 factorial arrangement of treatments was utilized with three body sizes at weaning and three floor space allowances. For each group, three blocks consisting of six pens each were randomly assigned to one of three pig size classifications (small, medium, or large). The largest one-third of gilts from each group were assigned to large, the next largest one-third of gilts assigned to medium, and the remaining one-third of gilts were assigned to small. Floor space

allowance treatments of 0.27(low density), 0.19 (normal density), and 0.15 (high density) m²/pig were randomly assigned within each block with two replicates per block. Different floor space allowances were achieved by placing eight, eleven, or fourteen pigs per pen, respectively. Two gilt pens in each room were left empty to be utilized as hospital pens for sick, injured, or weak pigs. The location of the hospital pens was randomly assigned to one of four positions on either end of the row or between body size blocks. When there were not enough gilts to completely fill a pen, barrows were placed in pens with the gilts to achieve the desired floor space allowances. Conversely, if there were excess gilts in a group, then extra pens were utilized on the barrow side and randomly assigned to size classification and floor space allowance so as to obtain data from the maximum number of gilts possible.

Body Weight Measurements

Initial BW were obtained at weaning (d 0) (Ohaus Defender 5000, Ohaus Corporation, Parsippany, NJ). Final BW were obtained at the end of the nursery phase (d 46) (VS-660, A and A Scales, LLC, Prospect Park, NJ).

Blood Sample Collection and Analyses

Gilts (n = 54) from Group 5 were randomly selected evenly across treatments (n = 3 per pen, n = 18 per floor space allowance) for blood collection on d 6 and d 40. The same animals were used for both collections. On each occasion, animals were placed supine on a v-board and approximately 7 mL of blood collected via jugular venipuncture using Vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ) into each of two tubes. One tube contained EDTA and the other had no additive. Blood containing EDTA was used for complete blood counts (CBC) conducted in the Toxicology laboratory at the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM, Blacksburg, VA). For blood collected into tubes containing

no additive, serum was harvested following centrifugation. One half of each serum sample was used for determination of the blood chemistry profile at VMRCVM. The other half of serum was used to determine cortisol concentrations using a commercially available RIA kit (Coat-A-Count; Siemens Diagnostic Products, Los Angeles, CA). Intra- and inter-assay CV were 6.7% and 13.2%, respectively, and the minimal detectable concentration was 2 ng/mL.

Necropsy

On d 53, 30 gilts (n = 10/floor space allowance) from Group 9 were randomly selected for necropsy. Animals were transported to the Virginia Tech Tidewater Agricultural Research and Extension Center (Suffolk) where the gilts were killed and the following weights and measures were recorded: BW, weight of the spleen, kidneys, liver, heart, uterus, and ovaries, vulva length, vulva width, and vulva area. The length and width of the vulva was determined using calipers.

Statistical Analyses

All data were analyzed using SAS (SAS Institute Inc., Cary, NC). Body weights at weaning and at the end of the nursery phase, total gain, and ADG were subjected to ANOVA using the GLM procedure. The model included treatment (i.e., floor space allowance), size category (small, medium, large), and their interaction as possible sources of variation with pen serving as the experimental unit. Blood chemistry values, CBC values, and serum cortisol concentrations were subjected to ANOVA for repeated measures using the Mixed procedure. The model included treatment, size, day (d 6 or d 40), and appropriate two- and three-way interactions as possible sources of variation. Pen served as the experimental unit for blood chemistry values and CBC values whereas pig served as the experimental unit for serum cortisol concentrations. Necropsy data were analyzed by ANOVA using the GLM procedure and the

model included treatment, size, and their interaction as possible sources of variation with individual pig serving as the experimental unit. Treatment effects were evaluated for significant linear and quadratic components.

Results

Growth Performance

Measures of growth performance are found in Table 1. Among treatments (floor space allowances), initial BW of gilts were similar (P=0.12) and as expected, BW among size classification were different (P<0.01) with large pigs weighing the most and small pigs, the least. There were effects of treatment (P<0.01) and size (P<0.01) on final BW and ADG. There were no effects of treatment \times size on these measures. At the end of the experiment, gilts allowed the greatest amount of floor space weighed more (P<0.05) than gilts allowed the intermediate or least amount of floor space. Gilts allowed the greatest amount of floor space also had the greatest ADG (P<0.01). Gilts classified as large had greater (P<0.05) final BW than medium gilts which had greater (P<0.05) final BW than small gilts. The ADG was greater (P<0.05) for large gilts than medium gilts, which had greater (P<0.05) ADG than small gilts.

Complete Blood Counts

Results for the CBC for different sized pigs allowed different amounts of floor space are found in Table 2. There were significant effects of floor space allowance for reticulocyte percentage (**RETIC%**), reticulocyte numbers (**RETIC#**), red blood cell distribution width (**RDW %**), number of red blood cells/100 white blood cells (**NRBC**) and tendencies for effects on mean corpuscular hemoglobin (**MCH**) and monocytes (**MONO**). Moreover, size of pig significantly affected red blood cells (**RBC**), hemoglobin (**HGB**), hematocrit (**HCT**), mean

corpuscular volume (**MCV**), RETIC%, RETIC#, banded neutrophils (**BAND**), and tended to affect white blood cells (**WBC**) and basophils (**BASO**). There were effects of day (P < 0.05) for RBC, RETIC%, RETIC#, NRBC, and plasma proteins (**PP**) and a tendency for an effect of day for HCT (P = 0.06) with values increasing from d 6 to d 40. In contrast, RDW%, WBC, neutrophils, segmented neutrophils (**SEG**), neutrophil to lymphocyte ratio (**N:L**), BASO, and platelets decreased from d 6 to d 40 (effect of day, P < 0.01).

There were treatment \times size \times day effects (P < 0.05) for neutrophils (Figure 1), SEG (Figure 2), and N:L (Figure 3). Neutrophil counts decreased from d 6 to d 40 in large gilts given the greatest amount of floor space (P = 0.05), medium gilts given the least amount of floor space (P < 0.05), and small gilts given intermediate floor space (tendency, P = 0.06), but not in the other groups (Figure 1). Medium gilts given the least amount of floor space (P < 0.05), large gilts given the greatest amount of floor space (P < 0.05), and small gilts given intermediate floor space (tendency, P = 0.07), but not gilts in the other groups, had greater SEG on d 6 than on d 40 (Figure 2). Medium gilts given the least amount of floor space (P < 0.05) and small gilts given intermediate floor space (tendency, P = 0.06), but not gilts in the other groups, had decreased N:L on d 40 compared to d 6 (Figure 3).

There were effects of treatment \times size (P < 0.05) on MCV (Figure 4), MCH (Figure 5), and RETIC% (Figure 6). There was a tendency for an effect of treatment \times size for RDW, NRBC, and BASO (data not shown). Small gilts given the least amount of floor space had greater (P = 0.02) MCV than small gilts given the greatest amount of floor space (Figure 4). Within floor space allowance, medium gilts given the least amount of floor space had lesser (P < 0.05) MCH than small gilts given the least amount of floor space (Figure 5). Medium gilts given the least amount of floor space tended (P = 0.09) to have lesser MCH compared to medium gilts

given intermediate floor space (Figure 5). Medium gilts given intermediate floor space had greater (P < 0.05) RETIC% compared to large gilts given intermediate floor space (Figure 6). Also, RETIC% tended (P = 0.08) to be greater for medium gilts given intermediate floor space than for medium gilts given the least amount of floor space. There was a tendency for RETIC % (P = 0.07) to be greater for small gilts given the least amount of floor space compared to medium and large gilts given the least amount of floor space (Figure 6).

Treatment × day affected (P = 0.03) BAND with gilts given the greatest amount of floor space tending to have greater values (P = 0.06) on d 6 than d 40 (Figure 7). Treatment × day also affected (P < 0.05) MONO and gilts given intermediate floor space had increased (P < 0.05) values on d 40 compared to d 6 (Figure 8).

Treatment × day tended (P = 0.07) to affect RETIC# and RETIC # increased from d 6 to d 40 for all treatment groups. Gilts given the greatest amount of floor space, however, tended (P = 0.07) to have the greatest RETIC# on d 40 compared to the other two groups (data not shown). An effect of size × day was detected for (P = 0.03) RETIC% (Figure 9). The RETIC% increased (P < 0.01) from d 6 to d 40 in small, medium, and large gilts. However, the RETIC% was less (P < 0.05) on d 40 for large gilts compared to medium and small gilts (Figure 9). Size × day affected (P = 0.03) RETIC# (Figure 10). Increases (P < 0.01) from d 6 to d 40 were detected for small, medium, and large gilts, however, large gilts had reduced (P = 0.01) RETIC# on d 40 compared to small and medium gilts (Figure 10).

Blood Chemistry Profile

Results for the blood chemistry profiles are found in Table 3. There were effects of treatment (P < 0.05) for calcium (**Ca**) and chloride (**Cl**) and a tendency for an effect of treatment (P = 0.09) on indirect bilirubin. Calcium concentration was less (P = 0.04) for gilts given the

least amount of floor space compared to gilts given the greatest and intermediate amounts of floor space. Calcium concentrations for gilts given the greatest amount of floor space and gilts given the intermediate amount of floor space did not differ (P = 0.98). Concentrations of Cl were greater (P = 0.05) for gilts given the least amount of floor space compared to gilts given intermediate floor space with gilts given the greatest amount of floor space having an intermediate value that did not differ from the other two groups. Concentrations of indirect bilirubin tended to be greater (P = 0.07) in gilts given the least amount of floor space compared to gilts given intermediate floor space; values for gilts given the greatest amount of floor space were intermediate and did not differ from the other two groups (P = 0.56).

There were effects of size of pig (P < 0.05) on urea nitrogen (**urea N**), creatinine, Ca, and albumin. Urea N was greater (P = 0.03) for small gilts than for both medium and large gilts. Creatinine was greater (P = 0.01) for large gilts compared to small gilts. Medium gilts were intermediate and did not differ from the other two groups (P = 0.34). Concentrations of Ca were greater (P = 0.03) for large gilts than for medium gilts; small gilts were intermediate and did not differ (P = 0.38) from the other two groups. Albumin was greater (P = 0.03) for large gilts compared to small gilts with medium gilts having an intermediate value that did not differ from the other two groups. Concentrations of glucose, P, Ca, Mg, total protein, albumin, globulin, gamma-glutamyltransferase, CK, Cl, and anion gap increased from d 6 to d 40 (effect of day, P < 0.05). There was a tendency for an effect of day (P = 0.07) for creatinine with greater values on d 40 compared to d 6. The concentration of AST/GOT decreased (P < 0.05) from d 6 to d 40 (effect of day, P < 0.05).

There were effects of treatment \times size (P < 0.05) for glucose and globulin concentrations (Figure 11). Globulin was greater (P < 0.05) for small gilts given intermediate floor space than

medium gilts given intermediate floor space, but there was no such relationship for gilts allowed the other amounts of floor space (Figure 11). Although there was an effect of treatment \times size (P < 0.05) on glucose concentrations, there were no effects of treatment within size classifications or size within treatment groups. There were effects of treatment \times day (P < 0.05) on aspartate aminotransferase/glutamic-oxalacetic transaminase (AST/GOT) (Figure 12), creatine kinase (CK) (Figure 13), and Cl (Figure 14). The AST/GOT decreased (P < 0.05) from d 6 to d 40 in gilts given intermediate floor space. Gilts given the least amount of floor space had greater AST/GOT on d 40 than gilts given intermediate floor space (Figure 12). Creatine kinase increased (P = 0.05) from d 6 to d 40 in gilts given the greatest amount of floor space (Figure 13). Chloride increased (P < 0.05) from d 6 to d 40 in gilts given the greatest and intermediate amounts of floor space (Figure 14). There was a trend (P = 0.08) for an effect of treatment \times day on globulin concentrations (data not shown). Globulin increased (P < 0.01) from d 6 to d 40 in gilts given the least amount of floor space only (from 2.1 to 2.6 ± 0.01 g/dL). Gilts given the least amount of floor space tended to have greater (P = 0.08) globulin on d 40 than gilts given the greatest amount of floor space (data not shown). There were effects of size \times day (P < 0.01) on glucose concentrations (Figure 15). Glucose increased (P = 0.02) from d 6 to d 40 in large, medium, and small gilts, however, on d 40, glucose concentrations were greater (P < 0.05) for medium gilts than large gilts with small gilts having an intermediate value not different from the other two groups (Figure 15).

Serum Cortisol

Concentrations of cortisol in serum for gilts of different sizes and housed with different floor space allowances are shown in Table 4. There were no main effects of size or floor space allowance. Across treatments cortisol concentrations increased from d 6 to d 40 (effect of day, *P*

< 0.05). There was an effect (P = 0.03) of treatment × size × day on serum cortisol concentrations (Figure 16). For medium-sized gilts, females given the greatest and least, but not intermediate, amount of floor space had increased (P < 0.01) cortisol concentrations from d 6 to d 40 (Figure 16). Compared to medium-sized gilts given intermediate floor space, medium-sized gilts given the greatest amount of floor space had greater (P < 0.01) concentrations of cortisol on d 40 (Figure 16). Small-sized gilts given the least amount of floor space had increased (P < 0.01) cortisol from d 6 to d 40 (Figure 16).

Necropsy

Organ measurements for large, medium, and small gilts housed at 0.27, 0.19, or 0.15 m^2/pig are shown in Table 5. For the subset of gilts necropsied, BW increased linearly with increased floor space allowance. Spleen weight (P < 0.01), uterus weight (P < 0.01), and ovary weights (P = 0.05) increased linearly with increased size of gilt. There were effects (P < 0.01) of treatment × size on liver weight (Figure 17). Large gilts given the greatest amount of floor space had greater (P < 0.01) liver weight than large gilts given either intermediate or the least amount of floor space (Figure 17). Medium gilts given the greatest amount of floor space tended (P = 0.07) to have greater liver weight than medium gilts given the intermediate amount of floor space. Within floor space allowances, medium-sized gilts given the greatest amount of floor space had greater (P < 0.01) liver weight than small gilts given the greatest amount of floor space. A tendency for an effect of treatment × size (P = 0.10) was detected for kidney weight (data not shown).

Discussion

The main objective of this study was to determine the effects of nursery floor space allowance on growth, physiology, and immunology in replacement gilts. Because the experiment

was conducted on a commercial farm it was impossible to alter size of the pens. Group size was changed (8, 11, or 14 gilts) to achieve the different floor space allowances. Thus, group size and floor space were confounded. Nevertheless, crowding achieved by increasing the number of gilts per pen during the nursery phase of rearing negatively impacted growth performance. Gilts in larger groups given less floor space had decreased ADG and decreased BW at the end of the nursery phase of production compared to gilts allowed more floor space. Also, gilts that were smaller upon entering the nursery barn had decreased ADG and decreased BW at the end of the nursery phase compared to gilts that were larger upon entry. These results are in accordance with other studies that have shown that decreased floor space allowance and increased group size results in decreased ADG. Gehlbach et al. (1966) found that growth to 50 kg was less for pigs reared at 0.18 m²/pig compared to 0.36 and 0.54 m²/pig. Wolter et al. (2002) reported that pigs housed at double stocking density (104 pigs/pen and 0.325 m²/pig) had less ADG and BW through 10 wk post weaning compared to pigs housed at single density (52 pigs/pen and 0.650 m²/pig). Although the design of the feeding system used in this study prevented us from determining feed consumption, it is widely accepted that decreased ADG due to crowding is a result of decreased ADFI (Kornegay and Notter, 1984; Lindemann et al., 1987). Lindemann et al. (1987) concluded that reduced ADFI and ADG were a result of decreased space per se and not a consequence of restricted feeder trough space.

At the end of the nursery phase of production, concentrations of total neutrophils, SEG, and N:L were decreased from initial concentrations for some floor space and size combinations.

Over time, large gilts given the greatest amount of floor space, and medium and small gilts given the least amount of floor space, showed decreased total neutrophils and SEG. Medium-sized gilts given the least amount of floor space and small gilts given intermediate floor space exhibited a

decreased N:L. These results agree with Friendship et al. (1984), who reported that SEG were greater in weanling pigs compared to feeder pigs. Banded and SEG make up total neutrophil concentrations, however, SEG concentrations greatly exceed BAND concentrations. In the current study, decreasing neutrophils and unchanging lymphocytes resulted in a decrease N:L. Gross and Siegel (1983) suggested that the heterophil (in the case of the pig, neutrophil) :lymphocyte ratio was a sensitive measure of stress in chicks and may be a better measure of long-term changes in the environment than changes in corticosteroid concentrations, which are more indicative of short-term changes (Gross and Siegel, 1983). An increasing ratio of neutrophils:lymphocytes is indicative of stress because neutrophils typically increase and lymphocytes typically decrease in response to various stressors. Kornegay et al. (1993b) did not observe an increase in N:L in response to decreased floor space in nursery pigs, although lymphocytes decreased by 13% from wk 3 to 6 post-weaning. Yen and Pond (1987) reported increased N:L at d 14 and 28 for pigs (7.5 kg BW) housed in groups of 16 (0.13 m²/pig) compared to groups of 8 (0.26 m²/pig). A subsequent study using the same housing conditions, however, produced no such effect. Gilts in the current study did not exhibit increased neutrophils, decreased lymphocytes, or an increased N:L indicating, perhaps, that the immune system was not stressed due to floor space restrictions.

It is generally assumed, however, that crowding induced by changes in stocking density causes stress to animals. Clearly, in the current study, crowding during the nursery phase of production did not affect serum concentrations of cortisol, the classical stress hormone. Cortisol concentrations increased with time from d 6 to d 40 in small- and medium-sized gilts given the least amount of floor space, but cortisol concentrations also increased from d 6 to d 40 in medium-sized gilts given greatest amount of floor space. The biological response to acute

stressors (parturition, castration, weaning, mixing of animals, restraint, transportation, slaughter) activates the hypothalamic-pituitary-adrenal axis and ultimately increases cortisol secretion. If the stress is chronic, then circulating levels of corticosteroids return to baseline even though the animal is still enduring a stressful situation (Mormede et al., 2007). Certain aspects of the physical and social environments to which farm animals, including nursery pigs are exposed, such as group size and floor space allowance, may be perceived as chronic stressors. The current results agree with other studies that showed restricting floor space did not consistently affect corticosteroid activity (Lindemann et al., 1987; Yen and Pond, 1987; Kornegay et al., 1993a; Marco-Ramell et al., 2011). Lindemann et al. (1987), however, did observe increased cortisol concentrations in pigs housed in groups of 10 compared to groups of 5.

Over time, AST concentrations decreased in gilts given intermediate floor space and gilts given the least amount of floor space had greater AST on d 40 than gilts given intermediate floor space. In contrast to these findings, Kornegay et al. (1993b) observed no effect of nursery floor space allowance on AST. In the study reported here, CK increased over time in gilts given the greatest amount of floor space. Kornegay et al. (1993b) also observed greater CK levels in pigs given greater amounts of floor space and attributed this to more traumatic musculoskeletal damage endured by these pigs. Creatine kinase is leaked into the serum proportional to the degree of damage that occurs to muscle cells (Kornegay et al., 1993b). Kornegay et al. (1993b) observed that pigs given less space moved and fought less frequently than pigs given more space. Necropsy revealed that pigs with adequate floor space actually suffered more traumatic injuries as evidenced by bruises and lameness.

Chloride concentrations increased from d 6 to d 40 for gilts given the greatest and intermediate amounts of floor space and glucose concentrations increased over time for all size

classifications. Increased circulating cortisol concentrations block entrance of glucose to cells and stimulate gluconeogenesis in the liver, causing an increase in plasma glucose levels (Barnett et al., 1983; Mormede et al., 2007). Thus, the increase over time in serum cortisol concentrations demonstrated in this study is consistent with a concurrent increase in plasma glucose. This finding is in agreement with Barnett et al. (1983) who observed increased glucose concentrations after a period of chronic stress due to unpleasant handling in gilts exposed to stressors 3 times weekly from 11 to 22 wk of age.

Crowding resulted in decreased Ca concentrations in this study. Lower levels of Ca and P in pigs housed with restricted floor space were suggested by Kornegay et al. (1993b) to be indicators of subtle stress. Corticosteroids decrease renal tubular absorption of Ca which, in turn, lowers serum Ca concentrations (Kornegay et al., 1993b). Persistently low serum Ca concentrations would stimulate the parathyroid gland to increase secretion of parathyroid hormone, which would increase excretion of P through the urine effectively lowering serum P (Kornegay et al., 1993b). Additionally, reduced Ca, P, and K in pigs housed with restricted floor space may have been from reduced feed intake.

Large and medium gilts given the greatest amount of floor space had greater liver weight than small gilts given the greatest amount of floor space. Also, large gilts given the greatest amount of floor space had greater liver weight than large gilts in the other treatment groups. Liver weight is generally proportional to BW, but varies by age and nutritional status (Yen, 2001). Of the gilts that were killed for necropsy, large gilts had greater BW than medium and small gilts and gilts given the greatest amount of floor space had greater BW than gilts given intermediate or least amounts of floor space. Thus, the increased liver weight for large gilts given

the greatest amount of floor space can be attributed to greater BW. Similar relationships were seen for spleen weight.

Floor space allowance did not influence weight of the uterus or ovaries. However, large gilts had greater weights of the uterus and ovaries than small and medium gilts. Uterine development in the pig is not dependent on ovarian steroids until 56 d after birth (Bazer et al., 2001). At the end of the nursery phase in the current study, pigs were approximately 70 d of age meaning uterine growth towards the end of the study was under the control of ovarian steroids. Because large gilts had greater ovary weights (possibly due to greater follicular development), perhaps increased secretion of ovarian steroids stimulated growth of the uterus.

Conclusion

Restricted floor space allowance significantly reduced growth rate during the nursery phase of production. There were inconsistent changes due to floor space allowance for concentrations of cortisol, the classical stress hormone which increases in response to acute stressors. Likewise, there were no observed differences between floor space allocations for N:L which is considered an indicator of long term stress. Instead, there were observed differences in blood constituents that are likely indicators of slight stress/ reduced feed intake and not indicators of seriously compromised health or immunity. Kim et al. (2008) found that total pigs born live in parity one was unaffected by treatments of nursery floor space allowance (P = 0.60). However, in parity two, total pigs born live (P = 0.24) and live pigs born/litter (P = 0.30) were numerically decreased by crowding stress. Future study involving the gilts from this experiment will determine the effects of floor space allowance in the nursery on subsequent sow performance and longevity.

Figures

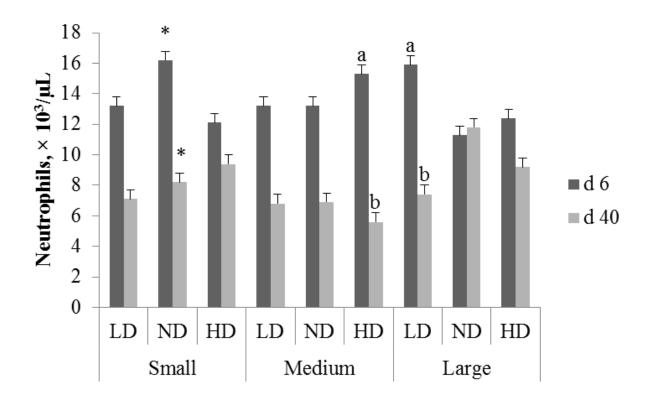


Figure 1. Effect of floor space allowance \times size \times day (P = 0.04) on total neutrophil concentrations for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within size and space allowance, means without a common superscript differ (P < 0.05); means with asterisks tend (P < 0.10) to differ.

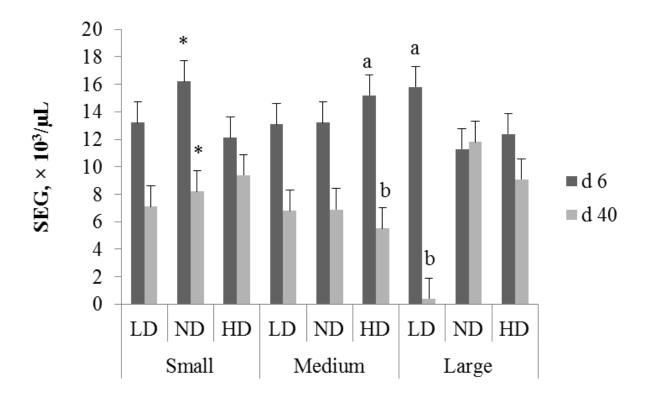


Figure 2. Effect of floor space allowance \times size \times day (P < 0.05) on segmented neutrophil (SEG) concentrations for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within size and space allowance, means without a common superscript differ (P < 0.05); means with asterisk tend (P = 0.07) to differ.

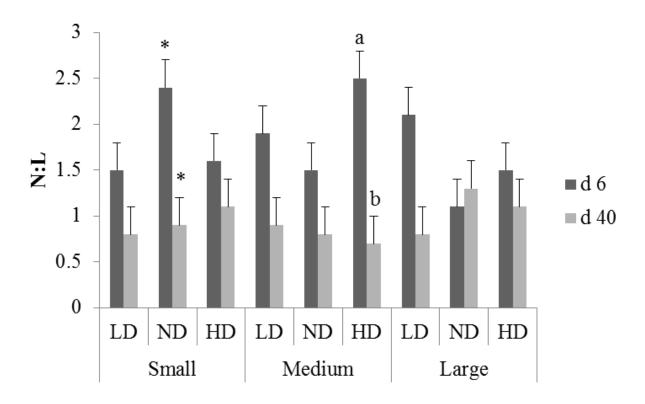


Figure 3. Effect of floor space allowance \times size \times day (P < 0.01) on neutrophil to lymphocyte ratio (N:L) for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within size and space allowance, means without a common superscript differ (P < 0.05); means with asterisk tend (P = 0.06) to differ.

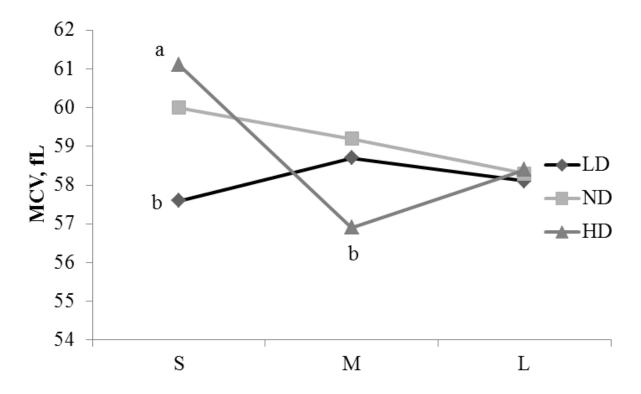


Figure 4. Effect of floor space allowance \times size (P < 0.05) on mean corpuscular volume (MCV) for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within a size category or space allowance, means without a common superscript differ (P < 0.05).

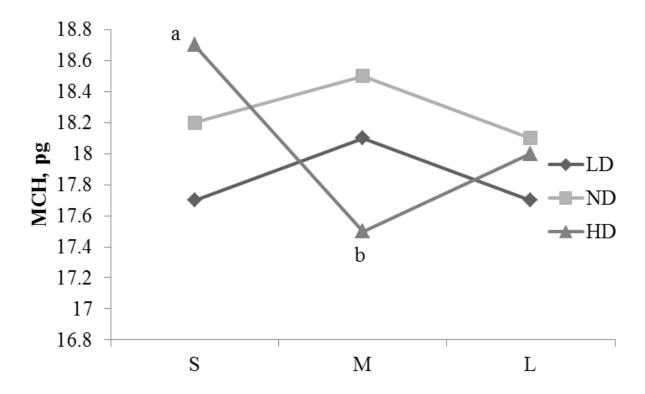


Figure 5. Effect of floor space allowance \times size (P < 0.05) mean corpuscular hemoglobin (MCH) for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within space allowance, means without a common superscript differ (P < 0.05).

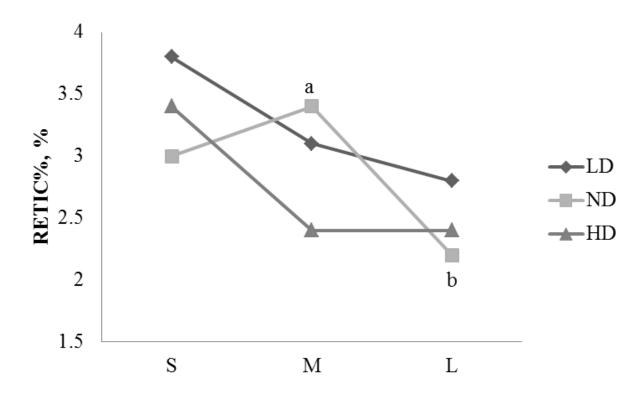


Figure 6. Effect of floor space allowance \times size (P < 0.05) on reticulocyte % (RETIC%) for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within a space allowance, means without a common superscript differ (P < 0.05).

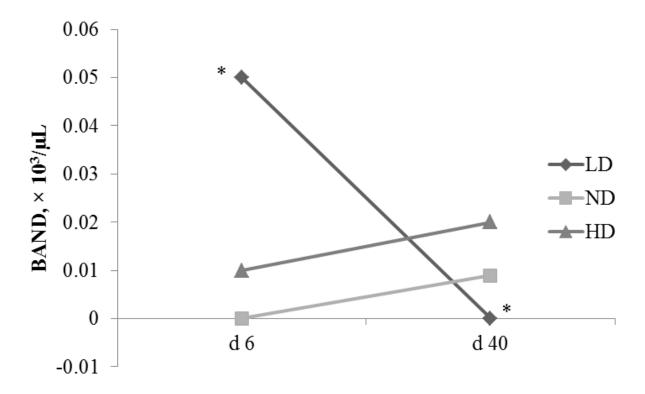


Figure 7. Effect of floor space allowance \times day (P = 0.03) on banded neutrophil (BAND) concentrations for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within space allowance, means without a common superscript differ (P < 0.05).

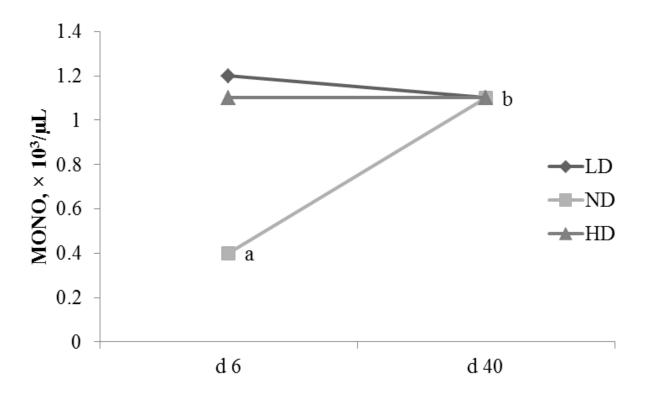


Figure 8. Effect of floor space allowance \times day (P < 0.05) on monocyte (MONO) concentrations for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within space allowance, means without a common superscript differ (P < 0.05).

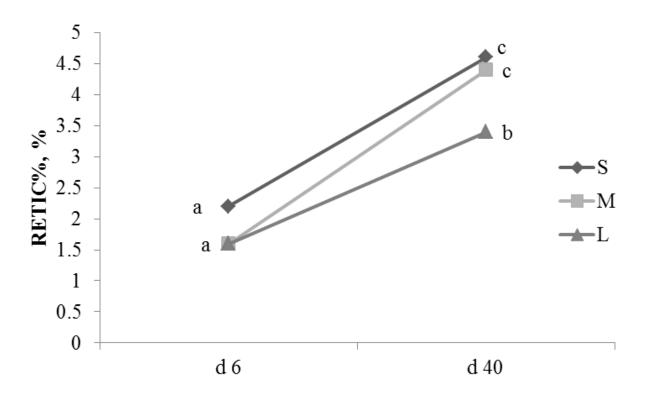


Figure 9. Effect of size \times day (P = 0.03) on reticulocyte percent (RETIC%) for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within day or size category, means without a common superscript differ (P < 0.05).

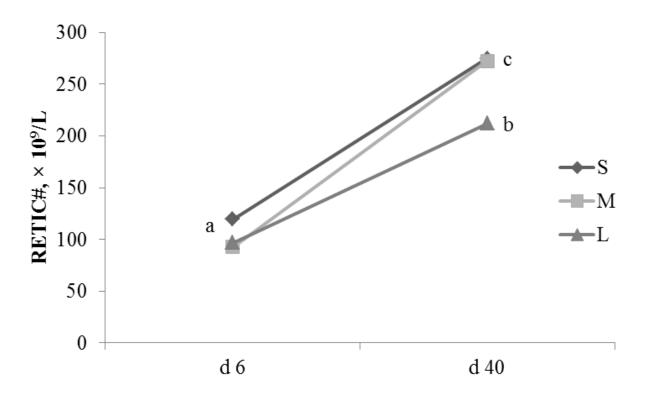


Figure 10. Effect of size \times day (P = 0.03) on reticulocyte number (RETIC#) for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within day or size category, means without a common superscript differ (P < 0.05).

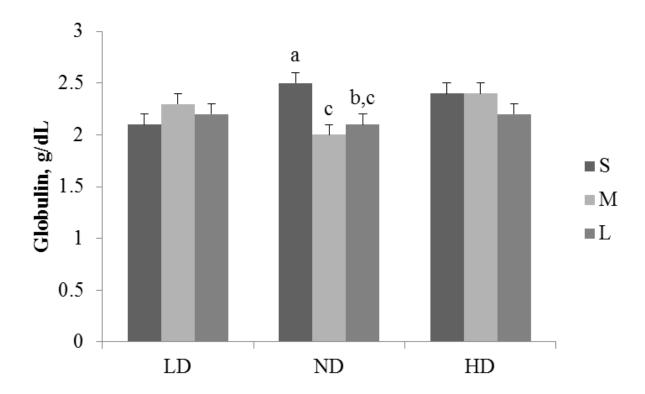


Figure 11. Effect of floor space allowance \times size (P = 0.02) on globulin concentrations for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within space allowance, means without a common superscript differ (P < 0.05).

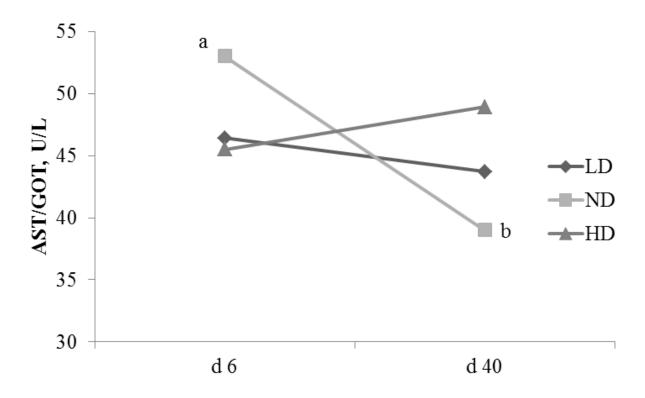


Figure 12. Effect of floor space allowance \times day (P < 0.01) on aspartate aminotransferase/glutamic-oxalacetic transaminase (AST/GOT) concentrations for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within space allowance, means without a common superscript differ (P < 0.05).

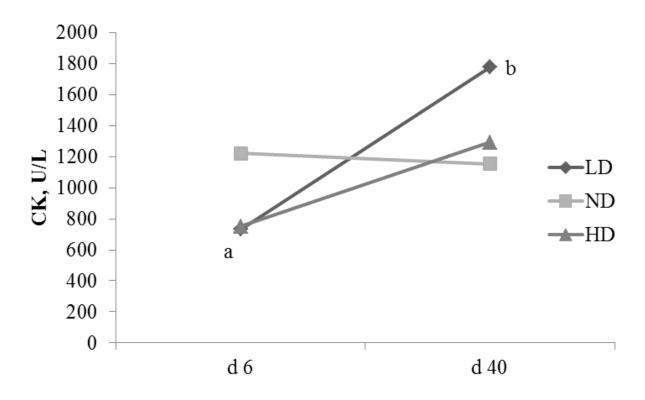


Figure 13. Effect of floor space allowance \times day (P = 0.03) on creatine kinase (CK) concentrations for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within space allowance, means without a common superscript differ (P < 0.05).

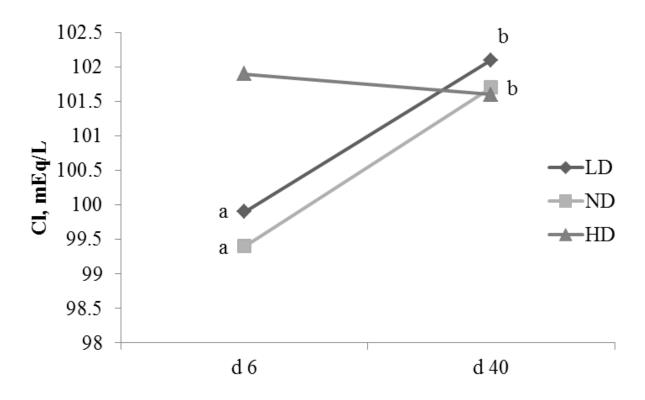


Figure 14. Effect of floor space allowance \times day (P = 0.02) on chloride (Cl) concentrations for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within space allowance, means without a common superscript differ (P < 0.05).

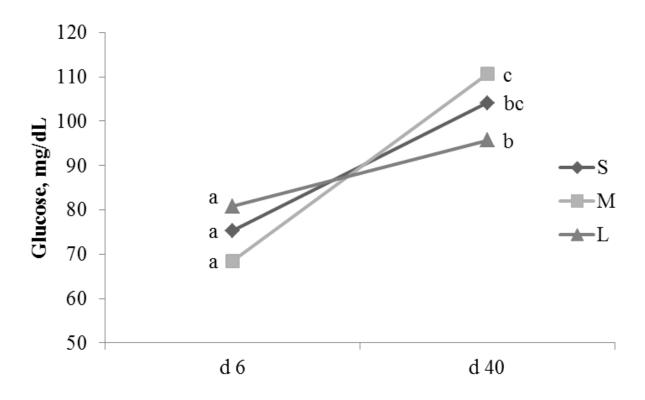


Figure 15. Effect of size \times day (P < 0.01) on glucose concentrations for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within size classification or day, means without a common superscript differ (P < 0.05).

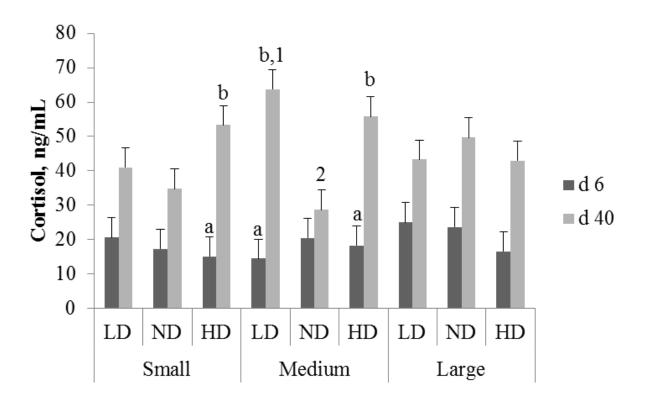


Figure 16. Effect of floor space allowance \times size \times day (P = 0.03) on serum cortisol concentrations for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within size classification and space allowance, means without a common alphabetical superscript differ (P < 0.05). Within size classification, means without a common numerical superscript differ (P < 0.05).

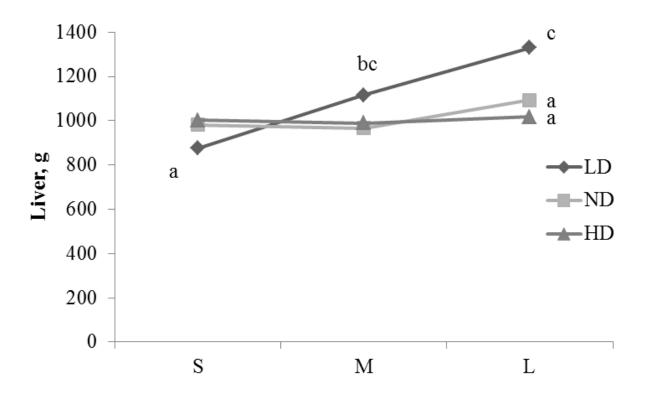


Figure 17. Effect of floor space allowance \times size (P < 0.01) on liver weight for small, medium, and large gilts housed at 0.15 (HD), 0.19 (ND), or 0.27 (LD) m²/pig during the nursery phase of production. Data are represented as least square means \pm SE. Within size classification or space allowance, means without a common alphabetical superscript differ (P < 0.05).

Tables

Table 1. Growth performance for small (S)-, medium (M)-, and large (L)-sized gilts housed at 0.15, 0.19, and 0.27 m^2/pig^1 . Values are LSMeans.

	Floor space (m ² /pig)						Size						
Item	0.15	0.19	0.27	SE	P	S	M	L	SE	P			
Initial BW, kg	5.73	5.68	5.54	0.07	0.12	4.43 ^a	5.60 ^b	6.92 ^c	0.07	< 0.01			
Final BW, kg	25.5^{a}	26.1 ^a	26.9^{b}	0.3	< 0.01	23.1 ^a	26.1^{b}	29.4 ^c	0.3	< 0.01			
Gain, kg	20.0^{a}	20.6^{a}	21.5 ^b	0.2	< 0.01	18.6 ^a	20.9^{b}	22.5^{c}	0.2	< 0.01			
ADG, kg/d	0.43^{a}	0.45^{a}	0.46^{b}	0.01	< 0.01	0.40^{a}	0.45^{b}	0.49^{c}	0.01	< 0.01			

¹Floor space allowances of 0.15, 0.19, and 0.27 m²/pig were achieved by placing in pens 14, 11, or 8 gilts, respectively. There were 82 pens with small pigs, 81 pens with medium pigs, and 80 pens with large pigs. There was a total of 1089, 823, and 625 gilts for the 0.15, 0.19, and 0.27 m² floor space allowances, respectively.

 $^{^{}a, b, c}$ Within a row for floor space allowances and sizes, means without a common superscript differ (P < 0.05).

Table 2. Complete blood counts for small (S)-, medium (M)-, and large (L)-sized gilts housed at 0.27 (n = 18), 0.19 (n = 18), and 0.15 (n = 18) m^2 floor space/pig. Values are LSMeans.

		Floo	or space (m ²	² /pig)		Size						
Item ¹	0.15	0.19	0.27	SE	P	S	M	L	SE	P		
RBC, $\times 10^6/\mu$ L	6.0	6.0	6.1	0.1	0.74	5.7 ^a	6.1 ^b	6.3 ^b	0.1	< 0.01		
HGB, g/dL	10.8	11.0	11.0	0.2	0.85	10.5 ^a	11.0 ^{ab}	11.2 ^b	0.2	0.02		
HCT, %	35.3	35.5	35.8	0.5	0.79	34.4 ^a	35.6 ^{ab}	36.5 ^b	0.5	0.03		
MCV, fL	58.8	59.2	58.1	0.4	0.15	59.5 ^a	58.3 ^{ab}	58.2^{b}	0.4	0.04		
MCH, pg	18.1	18.3	17.8	0.1	0.07	18.2	18.0	17.9	0.1	0.45		
MCHC, g/dL	30.7	30.9	30.7	0.2	0.52	30.5	31.0	30.8	0.2	0.19		
RETIC%, %	2.7^{a}	2.8^{ab}	3.2^{b}	0.1	0.03	3.4 ^a	3.0^{a}	2.5^{b}	0.1	< 0.01		
RETIC#, $\times 10^9$ /L	163.5 ^a	169.3 ^a	201.3^{b}	7.8	< 0.01	197.2 ^a	182.7^{a}	154.3 ^b	7.8	< 0.01		
RDW, %	17.8^{a}	16.4 ^b	17.3 ^a	0.3	< 0.01	17.0	17.6	16.9	0.3	0.13		
NRBC, /100 WBC	0.08	0.08	0.25	0.05	0.04	0.08	0.2	0.1	0.05	0.29		
WBC, $\times 10^3/\mu$ L	20.3	21.7	21.1	0.8	0.50	20.9	19.8	22.4	0.8	0.09		
Neutrophils $\times 10^3/\mu$ L	10.7	11.3	10.6	0.6	0.67	11.0	10.2	11.3	0.6	0.38		
SEG, $\times 10^3/\mu$ L	10.6	11.3	10.6	0.6	0.66	11.0	10.1	11.3	0.6	0.37		
BAND, $\times 10^3 / \mu L$	0.02	0.004	0.03	0.01	0.22	0.001 ^a	$0.03^{\rm b}$	0.01^{ab}	0.01	0.05		
LYMPH, $\times 10^3/\mu$ L	8.2	9.3	9.0	0.5	0.25	8.7	8.3	9.5	0.5	0.21		
N:L	1.4	1.3	1.3	0.1	0.82	1.4	1.4	1.3	0.1	0.84		
MONO, $\times 10^3/\mu L$	1.1	0.8	1.2	0.1	0.08	0.8	1.0	1.2	0.1	0.14		
EOS, $\times 10^3 / \mu L$	0.4	0.3	0.6	0.2	0.52	0.2	0.6	0.4	0.2	0.29		
BASO, $\times 10^3 / \mu L$	0.02	0.02	0.01	0.007	0.86	0.01	0.03	0.008	0.01	0.10		
Platelets, $\times 10^3 / \mu L$	554.9	491.3	539.4	38.3	0.49	566.6	539.4	479.6	38.3	0.28		
FIBR, mg/dL	211.1	230.6	219.4	20.0	0.79	238.9	213.9	208.3	20.03	0.53		
PP g/dL	5.7	5.6	5.6	0.09	0.64	5.6	5.7	5.7	0.09	0.86		

See text for definitions of abbreviations.

 $^{^{}a,b}$ Within a row for floor space allocations and pig size, means without a common superscript differ (P < 0.05).

Table 3. Blood chemistry profiles for small (S)-, medium (M)-, and large (L)-sized gilts housed at 0.27 (n = 18), 0.19 (n = 18), and 0.15 (n = 18) m^2 floor space/pig. Values are LSMeans.

	Floor space (m ² /pig)						Size						
Item ¹	0.15	0.19	0.27	SE	P	S	M	L	SE	P			
Glucose, mg/dL	86.7	90.9	90.1	2.1	0.34	89.9	89.5	88.3	2.1	0.85			
Urea N, mg/dL	14.2	14.5	14.0	0.4	0.66	15.3 ^a	13.6 ^b	13.8 ^b	0.4	< 0.01			
Creatinine, mg/dL	0.90	0.84	0.88	0.02	0.21	0.82	0.87	0.93	0.02	0.01			
Phosphorous, mg/dL	9.6	10.0	10.2	0.2	0.12	10.2 ^a	9.9^{ab}	9.8^{b}	0.2	0.37			
Calcium, mg/dL	10.2^{a}	10.5 ^b	10.4^{b}	0.07	0.02	10.4 ^{ab}	10.2^{a}	10.5 ^b	0.07	0.03			
Magnesium, mg/dL	2.5	2.5	2.6	0.04	0.21	2.5	2.5	2.5	0.04	0.79			
Protein tot, g/dL	4.8	4.7	4.7	0.09	0.41	4.7	4.7	4.8	0.08	0.54			
Albumin, g/dL	2.5	2.5	2.5	0.07	0.97	2.4 ^a	2.5 ^{ab}	$2.7^{\rm b}$	0.07	0.03			
Globulin, g/dL	2.3	2.2	2.2	0.06	0.15	2.3	2.2	2.1	0.06	0.20			
AST/GOT, U/L	47.2	46.0	45.2	1.8	0.70	46.4	48.7	43.1	1.8	0.11			
GGT, U/L	43.8	39.5	40.5	2.1	0.34	41.0	42.4	40.4	2.1	0.79			
Total Bili., mg/dL	0.21	0.11	0.14	0.04	0.18	0.13	0.14	0.19	0.04	0.51			
Direct Bili., mg/dL	0.003	0.0001	0.003	0.002	0.61	0.0001	0.003	0.003	0.002	0.61			
Indirect Bili., mg/dL	0.14	0.11	0.13	0.008	0.09	0.12	0.13	0.12	0.008	0.56			
CK, U/L	1020.6	1187.5	1253.5	137.4	0.48	1107.7	1106.1	1247.8	137.4	0.71			
Sodium, mEq/L	175.3	140.3	140.7	20.8	0.41	140.8	139.4	176.2	20.8	0.39			
Potassium, mEq/L	8.1	8.1	8.4	0.2	0.61	7.9	8.5	8.2	0.2	0.22			
Chloride, mEq/L	101.8^{a}	100.5 ^b	101.0^{ab}	0.3	0.05	100.8	100.9	101.6	0.3	0.17			
CO ₂ , mEq/L	24.1	25.3	25.0	0.6	0.38	24.4	25.4	24.6	0.6	0.49			
Anion Gap, mEq/L	22.3	22.7	22.2	0.7	0.88	23.3	21.8	22.0	0.7	0.28			

¹See text for definitions of abbreviations.

^{a,b}Within a row for floor space allocation and pig size, means without a common superscript differ (P < 0.05).

Table 4. Serum cortisol concentrations for small (S)-, medium (M)-, and large (L)-sized gilts housed at 0.27 (n = 18), 0.19 (n = 18), and 0.15 (n = 18) m^2 /pig. Values are LSMeans.

	Floor space (m ² /pig)					Size				
Item	0.15	0.19	0.27	SE	P	S	M	L	SE	P
Cortisol, ng/mL	33.6	29.1	34.7	2.3	0.20	30.3	33.5	33.5	2.3	0.54

Table 5. Organ measurements for small (S)-, medium (M)-, and large (L)-sized gilts housed at 0.27 (n = 10), 0.19 (n = 10), and 0.15 (n = 10) m^2/pig . Values are LSMeans.

		Floor spa	ace (m ² /pig)	Size						
Item	0.15	0.19	0.27	SE	P	S	M	L	SE	P
BW, kg	30.4	31.8	33.7	1.1	0.14	28.5 ^a	32.0 ^b	35.4°	1.1	< 0.01
Liver, g	1002.6^{a}	1014.0^{ab}	1107.3 ^b	33.1	0.07	953.8 ^a	1023.4 ^a	1146.8 ^b	33.1	< 0.01
Spleen, g	64.7^{a}	68.9^{ab}	$79.8^{\rm b}$	4.0	0.04	60.74 ^a	71.9^{ab}	$80.9^{\rm b}$	4.0	< 0.01
Kidneys, g	148.9	146.5	163.5	7.1	0.21	140.9 ^a	151.5 ^{ab}	166.4 ^b	7.1	0.05
Heart, g	174.5	164.4	163.5	12.8	0.80	155.9	168.0	178.5	12.8	0.47
Uterus, g	14.7	14.4	14.3	2.1	0.99	10.9 ^a	12.1 ^a	$20.4^{\rm b}$	2.1	< 0.01
Ovary, g	1.1	1.6	1.4	0.4	0.64	0.9	1.3	2.0	0.4	0.14
Vulva Length, mm	22.1	21.1	21.3	1.1	0.79	21.7	21.6	21.4	1.1	0.98
Vulva Width, mm	25.2	28.0	27.9	1.0	0.12	26.3	28.2	26.7	1.0	0.41
Vulva Area, mm ²	280.1	296.2	300.6	20.0	0.75	281.3	306.2	289.4	20.0	0.67

a,b,cWithin a row for floor space allocations and pig size, means without a common superscript differ (P < 0.05).

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