Title: Effect of weaning age and commingling after the nursery phase on humoral and behavioral indicators of well-being and on growth performance - **NPB #02-163**

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Abstract: Pigs from one farrowing group in which gilts were bred to farrow pigs that would be either 14 or 21 d of age at weaning, were divided into older and younger age groups (108 pigs/group) and penned 12 pigs/pen (1.5 × 3 m) in a wean-to-finish facility. At the end of the nursery phase, one-half of the pigs in each age group were removed, re-randomized, and commingled for the finishing phase, whereas the other half of the pigs remained in their original pens. Pigs were fed common Phase 1 (d 0 to 14) and Phase 2 (d 14 to 35) nursery diets, and fed a common four-phase program diet during growing/finishing with transitions at 45, 68, and 90 kg. The study was terminated when the lightest weight block averaged 107 lb. Plasma was obtained on d 0, 2, 10, 27, 37, 44, and 65 after weaning to determine leukocyte concentrations. Also, behavior was monitored during the nursery period at weaning, d 7, 14, and 27 post-weaning, and during the growing/finishing phase on d 35 (after commingling following the nursery phase), 38, 44, and 65 post-weaning. Older pigs were heavier throughout the nursery period; and the BW difference between younger and older pigs increased from 2 to 6.5 kg at the start and end of the nursery period. Older pigs had a greater concentration of white blood cells and lymphocytes on d 0, 2, and 10 post-weaning than younger pigs. Younger pigs spent less time lying recumbent on the day of weaning and more time standing or walking during the overall nursery phase, suggesting that younger pigs were less apt to settle into their new environment than older pigs. During Phase 3 (early finishing) and the overall finishing phase, younger pigs had greater ADG and G/F than older pigs. Moreover, during Phase 3, ADFI decreased when older pigs were commingled compared to older pigs that were not commingled, whereas there was no difference in ADFI of younger pigs, regardless of whether they were commingled or not. Unmixed pigs weaned at 14 d of age spent a greater percentage of time engaged in feeding behavior than mixed pigs weaned at 14 d of age, implying that young, mixed pigs greatly reduced feeding behavior in response to commingling compared to young, unmixed pigs. Results of this study indicate that weaning age affects growth performance in a wean-to-finish facility, as well as behavioral and immunological responses to weaning and commingling after the nursery phase. Weaning pigs at an early age results in a less immunologically-developed pig compared to pigs weaned later, but this may contribute to the benefits of early-weaning with respect to an overall improvement in gain and days to a common weight. However, management strategies should be further explored to optimize these benefits without the detrimental effects on health observed during the nursery period in this study.

Introduction: Pigs born and raised in conventional swine production systems are routinely weaned as early as 17 d of age, and as early as 12 to 14 d of age in off-site segregated early weaning systems (Maxwell and Carter, 2000). Although there are no legislative restrictions on weaning age in the U. S., the practice of weaning at an age earlier than 28 d of age is prohibited in Europe (European Community Commission Directive, 2001). Research has shown that early-weaning and removal of pigs to a second isolated site for rearing can reduce the potential for disease transfer from the dam (Harris, 1988). Pigs reared in isolation after weaning have been reported to have reduced immunological stress (Johnson, 1997), resulting in substantial improvements in growth and efficiency of feed utilization compared with those reared in conventional farrow-to-finish systems (Williams et al., 1997). Similarly, commingling pigs following the nursery phase is a common management practice in the swine industry, and imposes an additional stress upon the young pig. The advent of wean-to-finish facilities has potentially alleviated commingling after the nursery phase. However, the wasted space of placing weanling pigs in pens with space allowances for market hogs has led to the practice of double-stocking pens at weaning and later moving half of the pigs to another pen, which then introduces a commingling stress at an older age (Wolter et al., 2002). Results supporting wean-to-finish production systems in regards to piglet productivity are lacking, and the effect of these systems on pig well-being has not been evaluated. Therefore, the objective of this study was to determine the impact of age at weaning and of rearing pigs in the same pen in an all-in-all-out wean-to-finish facility vs. commingling pigs after the nursery phase on humoral and behavioral indicators of well-being and on growth performance.

Objectives:

1) Determine the effect of weaning age in a wean-to-finish facility on humoral and behavioral indicators of well-being and on growth performance.
2) Determine the effect of single pen wean-to-finish vs. commingling pigs following the nursery phase in a wean-to-finish facility on humoral and behavioral indicators of well-being and growth performance.
3) Determine the impact of weaning age and post-nursery commingling in a wean-to-finish facility on subsequent performance to market weight.

Materials and Methods:

a. *Allocation of Animals:* Pigs from one farrowing of 30 litters (DeKalb Line 348 gilts mated to EB sires) over a ten-day period were weaned when the average age was approximately 17 $\pm$ 0.26 d of age. Gilts were bred to farrow pigs that would average 14 and 21 d of age when weaned on the same day. Gilts were farrowed in crates in an environmentally-controlled farrowing room. Immediately after farrowing, pigs were ear-notched for individual identification, administered iron dextran, and tails were docked. Pigs were cross-fostered if necessary to equalize litters within their respective age groups. Pigs were divided into two age groups of equal number representing the older (21 d of age) and younger (14 d of age) groups of pigs (108 pigs in each group). Pigs within each age group were sorted into three categories based on weight and gender (36 pigs/block). Pigs from each gender and weight category within age group were then randomly allocated to pens within block in a double-stocked wean-to-finish facility (12 pigs/pen). This provided a total of 9 replications of each age group for the nursery study. At the completion of the nursery phase one-half of the pigs in each age category were removed from the double-stocked pens, re-randomized based on gender and weight, and commingled for the growing-finishing component of the study. One-half of the pigs remained in the same wean-to-finish pens. This arrangement of treatments permitted evaluation of the effects of weaning age in pigs double-stocked in a wean-to-finish facility as well as effects of post-nursery commingling on well-being and performance.

b. *Housing and Environment:* Pigs were housed in a wean-to-finish facility in totally slatted pens (1.52 m x 3.05 m; 0.39 m^2/pig) equipped with radiant heaters, a two-hole nursery feeder and a wean-to-finish cup waterer. Ambient room temperature was maintained at 78°F. In addition, a radiant heater oriented above rubber mats provided supplemental heat to a 1.8 m diameter area covering two pens/heater during the nursery phase. Wean-to-finish barns were curtain-sided and naturally ventilated, and contained a pit fan. The temperature was reduced gradually over the course of the nursery phase according to established procedures (no more than 3°F per week). The temperature was maintained at approximately 70°F throughout the starter and grower I phases and decreased to 68°F during the grower II and finisher phases. Water and feed were available ad libitum throughout the study.

c. *Experimental Management*: On the day of weaning, pigs were moved from the farrowing room and distributed to their assigned pen. Pigs were exposed to natural lighting, with supplemental electrical lighting provided only when animals were being observed. The pigs were offered ad libitum access to a Phase I nursery diet for the 0 to14 d period and a Phase II diet for the 14 to 35 d period (Table 1). On the day of completion of the nursery phase of the study, the pigs were started on the growing-finishing study. Pigs were fed a four-phase diet with transition from starter to grower I, from grower I to grower II and from grower II to finisher occurring when the mean weight of each block reached approximately 45, 68, and 90 kg, respectively (Table 2). All diets met or exceeded NRC (1998) requirements for all nutrients, and were formulated to simulate diets typical of those used in the swine industry. The study was terminated when the lightest block reached an average weight of 107 kg. The experiment was carried out in accordance with the Animal Care Protocol # 01015 for swine experiments issued by the University of Arkansas Animal Care Committee.

d. *Experimental Design:* Performance and behavioral data were analyzed as a 2 X 2 factorial design with two weaning ages and two post-nursery management systems, and pen as the experimental unit. Sampling over time was analyzed using the mixed model procedure of SAS, with day as the random variable. Analyses of variance for each day was performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).

Behavioral Measures

Behavioral and postural measures were recorded on day of weaning (0) and on d 7, 14, 27, and 35 (after commingling at the end of nursery phase), as well as d 38, 44 and 65 of the growing-finishing period. Prior to viewing, pigs were numbered 1 through 12 for behavioral observations during the nursery phase of the study (d 0, 7, 14, and 27 after weaning) and 1 through 6 after commingling (d 35, 38, 44, and 65 after weaning) with a permanent marker. Three pigs from each of the observed pens were randomly selected for viewing during the nursery phase, and another three randomly selected pigs were selected for viewing following the re-randomization after commingling. The following behaviors were recorded for each selected pig: 1) acts of aggression (biting, punching, head-thrusting, and chasing directed toward pen mates), 2) feeding, 3) drinking, 4) resting, 5) active (standing or locomotion), and 6) belly-nosing (Table 3).

Four pens per treatment were monitored with mounted camera (installed 2.3 m above floor level) surveillance equipment (Everplex 8CDX; Everfocus Electronics Corp., Pasadena, CA) for 24 hours to observe initial behaviors following weaning. Lenses of the eight cameras were 0.5 cm in diameter. Time-lapse videos (RCA standard grade, Socorro, TX) were recorded with a time-lapse video cassette recorder (Sanyo Electric Co., Ltd, Japan) in 12-h recording speed mode (7/60 second recording interval), and were viewed at a later date in two 1-hour increments (0900 to 1000 and 1400 to 1500). Duration of time spent by each pig engaged in these behaviors was recorded from 0900 to

1000 during the morning hour, and percentages of time were calculated based on the 2-hour observation.

Physiological Measures

A 5 mL blood sample was obtained from each pig via venipuncture on day 0, 2, 7, 13, 27, 37, 42, and 56. Blood samples were kept on ice for transport approximately 15 miles from the University of Arkansas Swine Research Unit to laboratory facilities at the University of Arkansas Animal Science Department. Blood parameters of interest (differential leukocyte proportions, lymphocyte-to-neutrophil ratio and granulocyte-to-monocyte ratios, and hematocrit) were obtained by analysis on an automated hematology analyzer (CELL-DYN® 3500SL System, Abbott, Abbot Park, IL) calibrated for porcine blood. An additional 15 mL blood sample was obtained on day 8 of the nursery period from 4 pigs randomly selected from 3 pens from each age group, and peripheral blood mononuclear cells were isolated to determine lymphocyte transformation, macrophage phagocytosis, and cytokine production (Interleukin (IL)-1, IL-10, Tumor necrosis factor-alpha and Interferon-gamma). This procedure was repeated on day 10 and 12 for the remaining 6 pens in each age group. Data from the three sampling days were pooled and designated as d 10 post-weaning. The same process was repeated post-nursery for all pens with 2 randomly chosen pigs per pen sampled, and pooled data was designated as d 44 post-weaning.

Performance Measures

Pigs were weighed as each block reached the projected weight for each phase. To obtain body weight, pigs were moved by pen into a weigh station and moved individually on the scale. Pig weights were averaged by pen and average daily gain (ADG) was calculated. Feed disappearance from each pen self-feeder was measured and average daily feed intake (ADFI) was calculated by pen as the difference between feed added and feed weighed back for each period. Gain:feed ratios for each period were calculated.

Results:

Behavioral Measures

Pigs weaned at 21 d of age spent a greater ($P < 0.05$) percentage of time resting on the day of weaning (d 0 post-weaning) than pigs weaned at 14 d of age (Table 4). Although the percentage of time spent active did not differ between pigs of different weaning ages at any observation day during the nursery period, pigs weaned at 14 d of age spent a greater ($P < 0.054$) proportion of time active during the overall nursery phase than pigs weaned at 21 d of age (Table 5). There was no difference in the percentage of time that pigs weaned at either age were engaged in aggressive behavior on any of the sampling days, but the frequency of times aggressive behaviors were observed were greater ($P < 0.05$) at weaning (d 0) than on any other observation day during the nursery period (Figure 1). During the growing/finishing period, the effect of weaning age and post-nursery commingling on feeding behavior was dependent upon the day of observation (weaning age x mixing x date interaction, $P < 0.05$; Figure 2). During the growing/finishing period, pigs that were weaned at 14 d of age and remained unmixed after the nursery period spent a greater ($P < 0.05$) percentage of time engaged in feeding activity on d 35 after weaning than 21 d old pigs that were mixed, or mixed pigs regardless of weaning age. The percentage of time spent feeding did not differ among pigs of either weaning age or post-nursery mixing treatment on day 38 or day 44 post-weaning; however, on d 65 post-weaning, pigs that were weaned at 21 d of age and mixed and pigs weaned at 14 d of age and unmixed spent a greater ($P < 0.05$) proportion of time engaged in feeding behavior than pigs in the other two treatments. Very few incidents of belly-nosing by pigs were observed in this study, and number and duration of belly-nosing did not differ between older and younger pigs.

Physiological Measures

There was no effect (P < 0.05) of weaning age or mixing after the nursery phase on the neutrophil:lymphocyte or granulocyte:monocyte ratio on any of the sampling days (Tables 6 through 12). On the day of weaning (d 0), pigs weaned at 21 d of age had a greater concentration of white blood cells than pigs weaned at 14 d of age. Specifically, pigs weaned at 21 d of age had greater (P < 0.05) concentrations of neutrophils and eosinophils, and eosinophils occupied a greater (P < 0.01) percentage of the total white blood cell concentration in older pigs compared to younger pigs (Table 6). On d 2 after weaning, pigs weaned at 21 d of age still had a greater (P < 0.01) concentration of white blood cells than pigs weaned at 14 d of age (Table 7). Specifically, older pigs had greater concentrations of neutrophils and lymphocytes than younger pigs, although there were no differences in these cells as a percentage of total white blood cells (Table 7). On d 10 after weaning, pigs weaned at 21 d of age had a greater (P = 0.057) concentration of white blood cells than pigs weaned at 14 d of age (Table 8). This was a result of older pigs having a greater (P = 0.016) concentration of lymphocytes than younger pigs. On d 27 after weaning, white blood cell concentrations did not differ between older and younger pigs; however, eosinophil concentrations and eosinophils as a percentage of total white blood cells were greater (P < 0.01) in older pigs than younger pigs (Table 9). The percentage of blood volume occupied by cells (hematocrit) was greater in younger pigs at weaning (Table 6) and on d 2 post-weaning (Table 7) than in older pigs; however, hematocrit was greater in older pigs on d 27 post-weaning (Table 9).

During the growing/finishing period, white blood cell concentrations on d 37 after weaning were increased (P = 0.04) when pigs weaned at 21 d of age were mixed and resorted compared to those remaining in their original pens, whereas there was no difference in white blood cell concentrations as a result of mixing when pigs were weaned at 14 d of age (interaction, P = 0.06; Table 10). The increase in white blood cell concentrations of older pigs that were mixed was a result of an increase in lymphocyte concentrations (interaction, P = 0.07). Regardless of whether the pigs were mixed and resorted or remained in original pens, hematocrit (19.02 $\pm$ 0.31 vs. 17.26 $\pm$ 0.31), eosinophil concentration (0.14 $\pm$ 0.01 vs. 0.09 $\pm$ 0.01) and eosinophils as a percentage of total white blood cells (2.10 $\pm$ 0.17 vs. 1.23 $\pm$ 0.17) were greater (P < 0.01) in older pigs than pigs weaned at 14 d of age. Moreover, on d 44 after weaning (Table 11), hematocrit (18.98 $\pm$ 0.12 vs. 17.79 $\pm$ 0.12), eosinophil concentration (0.08 $\pm$ 0.01 vs. 0.15 $\pm$ 0.01) and eosinophils as a percentage of total white blood cells (1.77 $\pm$ 0.11 vs. 0.97 $\pm$ 0.11) were greater (P < 0.01) in older pigs than pigs weaned at 14 d of age. On d 65 after weaning (Table 12), concentration of white blood cells (5.81 $\pm$ 0.51 vs. 4.43 $\pm$ 0.51) tended to be greater (P < 0.07) when pigs were weaned at 14 d of age compared to 21 d of age. Also, neutrophils as a percentage of total white blood cells (41.57 $\pm$ 4.23 vs. 29.13 $\pm$ 4.23) were greater (P < 0.05) in younger than older pigs, although older pigs had a greater (P < 0.05) percentage of lymphocytes (61.84 $\pm$ 3.67 vs. 51.34 $\pm$ 3.67) than younger pigs.

There was no (P < 0.05) effect of age at weaning or mixing on macrophage phagocytic capacity during the nursery or growing/finishing period (Table 13 and 14, respectively). Also, of the four cytokines profiled in this study, only interferon-γ was produced in cell culture at a detectable level, even though neither weaning age nor mixing after the nursery phase impacted the interferon-γ production in cell culture (Table 13 and 14). During the nursery period, pigs weaned at 14 d of age tended to have a greater (P < 0.10) lymphocyte proliferation response when peripheral blood mononuclear cells were stimulated with pokeweed mitogen (PWM) or concanavalin A (Table 13). Lymphocytes isolated during the growing/finishing period from pigs weaned at 14 d of age had greater (P < 0.05) proliferation (1576 $\pm$ 185 vs. 933 $\pm$ 185) in unstimulated cultures compared to older pigs (Table 14), and proliferation in response to PWM increased (P < 0.05) in lymphocytes isolated during the growing/finishing period when pigs weaned at 14 d of age were mixed compared to pigs weaned at 14 d of age that remained in their original pens. Conversely, proliferation decreased (P < 0.05) when older pigs were mixed compared to older pigs that remained in their original pens (interaction, P < 0.01).

Performance Measures

During the nursery phase of the experiment, pigs weaned at 21 d of age had greater (P < 0.01) ADG and ADFI during Phase 1, Phase 2, and in the overall nursery period than pigs weaned at 14 d of age (Table 15). Pigs weaned at 14 d of age were more efficient (P < 0.01) than pigs weaned at 21 d of age during Phase 2 of the nursery and throughout the overall nursery period (Phase 1 and 2). At the commencement of the experiment, pig body weight was greater (P < 0.01) when pigs were weaned at 21 d of age compared to pigs weaned at 14 d of age, and as expected the older pigs continued to be heavier (P < 0.01) throughout the nursery period; however, the difference in body weight increased from 2.15 kg at the initiation of the experiment, to approximately 6.5 kg at the end of the nursery period. In addition, nursery mortality was greater when pigs were weaned at 14 d compared to pigs weaned at 21 d of age (12% vs. 1%; Chi-square, P < 0.01).

During the growing/finishing phase of the experiment, ADG, ADFI and gain:feed did not differ between pigs weaned at 21 or 14 d of age during Phase 1, Phase 2, or Phase 4 (Table 16). However, during Phase 3, pigs weaned at 14 d of age had greater (P < 0.05) ADG, ADFI, and gain:feed, and greater (P < 0.01) ADG and gain:feed in the overall growing/finishing period, than pigs weaned at 21 d of age. The removal and commingling of one half of the pigs from each pen at the end of the nursery period and resorting for the growing/finishing phase of the experiment did not affect ADG, ADFI, gain:feed, or pig body weight (Table 17). However, ADFI decreased during Phase 3 (P < 0.05) when pigs weaned at 21 d of age were mixed and resorted compared to those that remained in original pens, while there was no difference in ADFI of pigs weaned at 14 d of age, regardless of whether they were mixed and resorted or were left in their original pens (interaction, P = 0.08; Figure 3). Mixing and resorting pigs following the nursery phase of the study had no effect on the number of days for pigs to reach a common market weight of 230 lb. However, age of pigs at weaning did impact days to market, such that pigs weaned at 14 d of age reached a common weight of 230 lb four days sooner than pigs weaned at 21 d of age (143.9 vs. 148.4 $\pm$ 1.3 d; P < 0.05; Figure 4)

Discussion:

Behavioral Measures

The incidence of belly-nosing was not affected by age of pigs at weaning in this study, although many studies have reported a greater incidence of belly-nosing when pigs are weaned at 14 d of age (Metz and Gonyou, 1990; Gonyou et al., 1998, Worobec et al., 1999). However, the observation times in this study may have overlooked aberrant behaviors that may have occurred in either age group. Moreover, there was no evidence of lesions present on pigs as a result of repetitive belly-nosing behavior, indicating that belly-nosing activity was likely minimal.

Behavioral observations in this study indicate that younger pigs spent less time resting on the day of weaning and more time standing or walking during the overall nursery phase, suggesting that younger pigs were less apt to settle into their new environment than older pigs. Worobec et al. (1999) reported similar findings in pigs weaned at 7 and 14 d of age compared to pigs weaned at 28 d of age, in which younger pigs exhibited more escape behaviour, less interaction with neighboring pigs and less time feeding than pigs weaned at an older age. This nervousness and unrest may have contributed to the lower weight gains observed in young pigs compared to older pigs in the nursery phase of the study, even though a disease challenge likely contributed to the decrease in gain as well.

During the growing-finishing period, differences in the percentage of time spent engaged in feeding behavior during the growing/finishing period was dependent on the age of pigs at weaning, whether pigs were mixed after the nursery period, and the time after mixing occurred. Mixing decreased the percentage of time pigs engaged in feeding behavior directly after the mixing event for both age groups. This suggests that the reestablishment of the social structure within pens was necessary when pigs were regrouped. This response was transient, with no observed differences in duration of feeding behavior in the days following the initial post-nursery mixing, except on d 65 after weaning. Similarly, other research has reported that altered or agnostic behaviors were present only during a short time period after the mixing stress (McGlone et al., 1987; Heetkamp et al., 1995). However, we have no explanation for the observations on d 65 after weaning, in which pigs weaned at 21 d of age and mixed and pigs weaned at 14 d of age and remaining unmixed spent a greater percentage of time engaged in feeding behavior, although this was observed at the beginning of the Phase 2 time period that feed intake and gain of pigs weaned at 14 d of age began to increase.

Physiological Measures

Within all production systems pigs are exposed to varying degrees of stress, through processing practices, handling and movement or weaning from their dam. Stress is typically measured by evaluating changing levels of serum cortisol as an indicator of activation of the hypothalamic-pituitary-adrenal axis in response to a defined stressor (Dantzer and Mormede, 1983). Although cortisol levels have consistently been reported to elevate in response to stress, the very act of obtaining the blood sample intrinsically alters cortisol response (McGlone et al., 1993). It has been suggested that immunological evaluations, such as the neutrophil:lymphocyte ratio in the blood, may be a more reliable measure of stress than cortisol concentration (Gross and Siegel, 1983; Stull et al., 1999). Additional research evaluating the effects of stress on porcine immunity indicates that immune measurements, such as lymphocyte proliferation, macrophage phagocytosis, and the production of several cytokines, are altered in response to stress in the pig (Morrow-Tesch et al., 1994; Edfors-Lilja et al., 2000).

Although the white blood cell concentrations were similar between younger and older pigs on d 27 after weaning, indicating the younger pigs may be advancing in their immunological development, younger pigs were less immunologically responsive to the stress of mixing and resorting after the nursery phase than older pigs as indicated by the interaction of weaning age and mixing treatment observed 3 days after the resorting. White blood cell concentrations of older pigs that were mixed and resorted increased compared to older pigs that remained in their original pens, whereas white blood cell concentrations of younger pigs did not respond to being resorted. Although young pigs did not respond immunologically to commingling, the difference in the percentage of time engaged in feeding behavior was very pronounced between mixed and unmixed pigs weaned at 14 d of age, indicating that young mixed pigs greatly reduced feeding behavior in response to commingling compared to young unmixed pigs.

Physiological responses to stress and behavior involve complex interactions of the central nervous system, the endocrine system, and the immune system (Yang and Glaser, 2000), and these systems all influence health and well-being in response to environmental and management conditions. In an experiment evaluating the effects of stress at birth on cortisol concentrations, pigs responded to stress associated with a Caesarian-section birth with elevated serum cortisol, although this increase was not associated with a resulting increase in ACTH, suggesting a disregulation of ACTH and cortisol in Caesarian-derived pigs (Daniel, 1999). Hohenshell et al. (2000) reported a similar disregulation of the hypothalamic-pituitary-adrenal axis between the pituitary and the adrenal gland in early-weaned pigs that was not present in late-weaned pigs. Although not directly correlated with the sensitivity of the immune system in response to stressors, this disregulation of endocrine function could be a cause of the inability of early-weaned pigs to respond immunologically to the mixing and resorting stress during the post-nursery period in the present study.

Whereas the results of this study show that management conditions perceived as stressors, in this case, weaning and commingling pigs after the nursery period, do elicit an immune response and influence behavior, there is no indication that any one measure of the immune response or behavior can quantify well-being in pigs. In this study, stress induced a spike in the white blood cell concentration in older pigs, which can also be indicative of an inflammatory challenge from disease. While there was a tendency for mixed pigs to have higher concentrations of neutrophils, older pigs that were mixed and resorted also responded with elevated concentrations of lymphocytes; however this immune response could not be associated with growth performance or any aggressive behavior observations. A stress-associated increase in cortisol can result in an alteration of blood leukocyte concentrations, specifically an increase in neutrophils and a concomitant decrease in lymphocytes in the peripheral blood, that would result in an increase in the neutrophil-to-lymphocyte and granulocyte-to-monocyte ratio in the stressed animal (McGlone et al., 1993; Gross and Siegel, 1983). In the present study, neutrophil-to-lymphocyte and granulocyte-to-monocyte ratios were similar among pigs, regardless of weaning age or mixing treatment, even though the non-significant increase in the concentration and percentage of monocytes in response to mixing on d 37 (two days following the commingling event) is interesting to note. To further compound the difficulty in measuring the immune response or behavior as indications of welfare, there were very few differences in behavioral responses between pigs weaned at different ages or between pigs mixed after the nursery phase and those that remained unmixed, and immune responses were inconsistent depending upon the age of the pig at weaning. Whereas the results of this study raise interesting questions about the effect of management environments and stressors on pigs weaned at varying ages on subsequent performance and health, no single measure of growth performance, immunity, or behavior was found to conclusively measure a pig's welfare in response to wean-to-finish management schemes.

Performance Measures

The lower growth rate during the nursery period of pigs weaned at 14 d of age compared to older pigs is in contrast to other studies that reported either an improvement in ADG of early-weaned (10 d) pigs compared to late-weaned (30 d) pigs (Hohenshell et al., 2000) or no effect of weaning age on rate of the growth in the overall nursery period (Dritz et al., 1996). Although Hohenshell et al. (2000) reported greater ADG in early-weaned pigs at 42, 65, and 102 d of age compared to late-weaned pigs, early-weaned pigs were fed a nutrient-rich starter diet until the late-weaned pigs were moved into the nursery, which may have altered their subsequent performance compared to older pigs. Because sows in the present study were bred in the same group to farrow pigs that would average either 14 or 21 d at weaning, pigs were weaned on the same day to the same wean-to-finish facility and fed the same diet. Thus, the effect of weaning age should not be confounded by health status of the sow herd, environmental conditions, or management practices. To our knowledge this is the first experiment to report that early-weaned pigs overcame a deficit in body weight at the end of the nursery period to reach a common market weight in less days than pigs weaned at 21 d of age. Others have reported no effect of weaning age when evaluating overall gain from birth to market weight (Hohenshell et al., 2000) and no difference in BW when comparing early- and late-weaned pigs at a common age (Dritz et al., 1996).

The difference in mortality between the young and old pigs during the nursery period is interesting to note and supports the reported immune suppression observed in pigs weaned at 2 to 3 weeks of age (Blecha et al., 1983). Of the pigs that died during the nursery phase, all exhibited signs of *Steptococcus suis* and the majority were pigs weaned at 14 d of age. Pigs weaned at a younger age, likely had a less developed, more naive immune system that was unable to respond to the disease challenge. This is supported by the greater concentration of white blood cells observed in older pigs compared to younger pigs initially at weaning as well as d 2 and d 10 after weaning.

In conclusion, the results of this study indicate that weaning age affects growth performance in a wean-to-finish facility, as well as behavioral and immunological responses to weaning and commingling after the nursery phase. Weaning pigs at an early age results in a less immunologically-developed pig compared to pigs weaned later, and this may contribute to the benefits of early-weaning with respect to an overall improvement in gain and days to a common weight. However, management strategies should be explored further to optimize these benefits without the detrimental effects on health, as observed during the nursery period in this study.

Lay Interpretation: Age at weaning impacts growth performance as well as behavioral and immunological responses to management stressors, such as weaning and commingling after the nursery phase. A study conducted at the University of Arkansas evaluating the effects of weaning age and commingling after the nursery phase in wean-to-finish facilities on growth performance, immunological measurements, and behavioral observations, reported that older pigs were heavier throughout the nursery period. Body weight difference between younger and older pigs increased from 4.4 to 13.2 lb at the start and end of the nursery period, respectively. Younger pigs seemed to be more active (standing or walking vs. lying recumbent) than older pigs, and less immunogically developed, as evidenced by the increase in mortality rate observed for younger pigs during the nursery phase. However, pigs weaned at 14 d of age grew faster during the growing/finishing period and reached a common body weight (230 lb) four days sooner that pigs weaned at 21 d of age. It is unclear why the pigs weaned at 14 d of age that survived the nursery phase responded so robustly in the subsequent growing/finishing phase, although differences in immune system development may be the cause.

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Table 1. Composition of Phase 1 (d 0 to 14) and Phase 2 (d 10 to 35) diets fed to pigs during the nursery phase (as-fed).

Item, %	Phase 1	Phase 2
Yellow corn	27.82	46.51
Steam rolled oats	12.00	5.00
Dried whey	20.50	8.00
Lactose	-	5.00
Soy protein concentrate	8.00	-
Soybean meal, 48% CP	11.00	20.00
Spray-dried plasma protein	6.00	1.75
Select menhaden fish meal	8.00	7.00
Soybean oil	4.00	-
Fat		4.00
Ethoxyquin	0.03	0.03
Lysine HCl	0.15	0.17
Threonine	0.08	0.07
Methionine	0.10	0.07
Isoleucine	0.02	-
Antibiotic [a]	0.10	0.25
Mineral premix[f]	0.15	0.15
Vitamin premix	0.25	0.25
Zinc oxide	-	0.25
Copper sulfate	-	0.07
Dicalcium phosphate	1.12	0.65
Calcium carbonate	0.38	0.38
Salt	0.30	0.40

[a]Neo-Terramycin and Mecadox was provided in the diets during Phase 1 and Phase 2, respectively.

Table 2. Composition of Phase 1, Phase 2, Phase 3, and Phase 4 diets fed to pigs during the growing/finishing period (as-fed).

Item, %	Phase 1	Phase 2	Phase 3	Phase 4
Yellow corn	71.865	75.10	80.00	83.19
Soybean meal, 48% CP	23.00	18.10	13.50	10.50
Fat	2.30	4.00	4.00	4.00
Ethoxyquin	0.03	0.03	0.03	0.03
Lysine	0.15	0.15	0.15	0.15
Threonine	0.02	0.02	0.02	-
Dicalcium phosphate	0.94	0.95	0.75	0.755
Calcium carbonate	0.77	0.80	0.70	0.60
Mineral premix[b]	0.15	0.15	0.15	0.125
Vitamin premix[c]	0.15	0.15	0.15	0.125
Tylosin-40	0.125	0.05	0.05	0.025
Salt	0.50	0.50	0.50	0.50

Table 3. Observed behaviors and their definitions.[1]

Behavior	Definition
Resting	Lateral and sternal recumbancy
Feeding	Head positioned in feeder
Drinking	Snout in contact with nipple waterer
Aggression	Biting, pushing, head-thrusting, and chasing directed toward penmates (McGlone, 1986)
Belly-nosing	Repetitive rooting movement with the snout on another piget (Fraser, 1978)

[1]Definitions for observed behaviors excerpted from Gardner et al., 2001.

Table 4. Behavioral data (presented as percentage of time engaged in each respective behavior) collected on each of four observation days during the nursery phase from pigs weaned at either 14 or 21 d of age.

	Observation days								
	Weaning (d 0)		d 7 post-weaning		d 14 post-weaning		d 27 post-weaning		
Age at weaning:	14	21	14	21	14	21	14	21	SE
Observation, %									
Resting	26.2[b]	45.5[a]	32.5	36.6	34.2	37.9	48.3	63.2	6.4
Active	32.8	32.0	35.4	29.6	25.8	21.4	27.7	13.5	5.2
Drinking	1.9	3.2	1.3	1.5	1.5[b]	4.5[a]	1.8	1.1	0.80
Feeding	1.8	0.0	9.1	8.9	12.6	11.5	13.3	7.6	2.9
Aggression	3.1	3.3	3.7	1.8	1.8	1.0	1.9	2.0	1.6

[a,b] Means within each action in each observation day with differing superscripts are different ($P < 0.05$).

Table 5. Summary of behavioral data (presented as percentage of time engaged in each respective behavior) collected during the overall nursery phase from pigs weaned at either 14 or 21 d of age.

	Age at weaning, d			
Observation, %	14	21	SE	P=
Resting	36.1	46.1	4.00	0.130
Active	30.1	22.6	2.23	0.054
Drinking	1.7	2.5	0.47	0.255
Feeding	11.2	9.1	1.69	0.399
Aggression	2.9	2.1	0.61	0.387

Table 6. Differential leukocyte concentrations and leukocytes as a percentage of total white blood cells (WBC) on the day of weaning (d 0) from pigs weaned at 14 or 21 d of age.

	Weaning age			
× 10^3/μL	14 d	21 d	SE	P-value
WBC	2.83	3.51	0.17	0.012
Neutrophils	0.94	1.40	0.11	0.012
Lymphocytes	1.56	1.82	0.11	0.097
Monocytes	0.07	0.10	0.03	0.457
Eosinophils	0.03	0.06	0.01	0.002
% WBC				
Neutrophils	33.54	35.94	2.36	0.480
Lymphocytes	55.60	54.37	1.16	0.466
Monocytes	1.95	3.19	0.81	0.290
Eosinophils	1.08	1.59	0.11	0.004
Neu/lym ratio	0.63	0.76	0.06	0.15
Gran/mono ratio	124.96	203.19	40.49	0.19
Hematocrit, %	13.75	13.03	0.22	0.033

Table 7. Differential leukocyte concentrations and leukocytes as a percentage of total white blood cells (WBC) on d 2 after weaning from pigs weaned at 14 or 21 d of age.

	Weaning age			
× 10^3/μL	14 d	21 d	SE	P-value
WBC	2.80	3.92	0.19	0 .001
Neutrophils	1.15	1.68	0.16	0.036
Lymphocytes	1.44	2.03	0.14	0.011
Monocytes	0.06	0.08	0.03	0.691
Eosinophils	0.03	0.04	0.01	0.596
% WBC				
Neutrophils	40.34	42.13	2.94	0.672
Lymphocytes	52.13	51.47	2.03	0.820
Monocytes	2.07	1.94	0.74	0.896
Eosinophils	1.13	1.00	0.13	0.526
Neu/lym ratio	0.86	1.11	0.11	0.115
Gran/mono ratio	138.35	248.81	57.93	0.196
Hematocrit, %	13.59	12.66	0.21	0.006

Table 8. Differential leukocyte concentrations and leukocytes as a percentage of total white blood cells (WBC) on d 10 after weaning from pigs weaned at 14 or 21 d of age.

	Weaning age			
× $10^3/\mu L$	14 d	21 d	SE	P-value
WBC	5.56	6.53	0.33	0.057
Neutrophils	1.90	1.47	0.40	0.455
Lymphocytes	3.03	4.24	0.32	0.016
Monocytes	0.04	0.02	0.02	0.335
Eosinophils	0.03	0.03	0.01	0.661
% WBC				
Neutrophils	32.85	21.94	5.82	0.204
Lymphocytes	54.86	64.29	4.03	0.117
Monocytes	0.83	0.33	0.26	0.194
Eosinophils	0.58	0.56	0.11	0.917
Neu/lym ratio	1.13	0.67	0.21	0.137
Gran/mono ratio	259.23	143.11	60.40	0.193
Hematocrit, %	15.34	15.88	0.32	0.253

Table 9. Differential leukocyte concentrations and leukocytes as a percentage of total white blood cells (WBC) on d 27 after weaning from pigs weaned at 14 or 21 d of age.

	Weaning age			
× $10^3/\mu L$	14 d	21 d	SE	P-value
WBC	8.92	8.82	0.29	0.821
Neutrophils	4.06	3.76	0.23	0.356
Lymphocytes	4.46	4.71	0.30	0.560
Monocytes	0.08	0.04	0.02	0.063
Eosinophils	0.10	0.20	0.02	0.001
% WBC				
Neutrophils	45.37	42.59	2.61	0.463
Lymphocytes	50.14	53.16	2.07	0.318
Monocytes	0.93	0.47	0.17	0.077
Eosinophils	1.18	2.26	0.16	0.001
Neu/lym ratio	0.98	0.93	0.08	0.628
Gran/mono ratio	156.32	186.99	17.53	0.234
Hematocrit, %	16.51	17.41	0.20	0.005

Table 10. Effect of mixing and age at weaning on peripheral blood leukocyte concentrations and proportions of pigs on day 37 after weaning.

	Weaning age = 14		Weaning age = 21			P-value		
× $10^3/\mu L$	Mixed	Unmixed	Mixed	Unmixed	SE	Age	Mixed	Interaction
WBC	7.81[a]	7.70[a,b]	8.56[a]	5.95[b]	0.65	0.45	0.04	0.06
Neutrophils	2.94	2.39	3.35	2.42	0.40	0.59	0.07	0.63
Lymphocytes	4.37[a,b]	4.80[a]	4.55[a]	3.13[b]	0.49	0.14	0.32	0.07
Monocytes	0.35	0.05	0.36	0.03	0.23	0.99	0.17	0.94
Eosinophils	0.10	0.07	0.15	0.14	0.02	<0.01	0.19	0.68
% WBC								
Neutrophils	37.97	32.94	37.64	38.82	4.31	0.52	0.66	0.48
Lmphocytes	57.13	60.91	55.09	53.36	3.39	0.17	0.76	0.42
Monocytes	2.52	0.64	2.41	0.41	1.43	0.90	0.19	0.97
Eosinophils	1.44	1.02	1.94	2.26	0.24	<0.01	0.83	0.14
Neu/lym ratio	0.78	0.62	0.81	0.86	0.11	0.21	0.61	0.34
Gran/mono ratio	119.30	100.56	123.98	110.00	17.62	0.69	0.36	0.89
Hematocrit, %	16.79	17.73	19.08	18.97	0.44	<0.01	0.35	0.24

[a,b] Means with different superscripts are significantly different ($P < 0.05$)

Table 11. Effect of mixing and age at weaning on peripheral blood leukocyte concentrations and proportions of pigs on day 44 after weaning.

	Weaning age = 14		Weaning age = 21			P-value(≤)		
× $10^3/\mu L$	Mixed	Unmixed	Mixed	Unmixed	SE	Age	Mixed	A x M
WBC	9.25	8.42	9.35	8.38	0.61	0.96	0.15	0.92
Neutrophils	4.13	3.72	4.03	3.83	0.46	0.99	0.51	0.82
Lymphocytes	4.67	4.30	4.84	3.92	0.40	0.79	0.12	0.49
Monocytes	0.14	0.03	0.03	0.08	0.07	0.68	0.65	0.23
Eosinophils	0.08	0.08	0.13	0.17	0.01	0.01	0.23	0.16
% WBC								
Neutrophils	45.14	43.15	42.00	43.19	3.79	0.69	0.92	0.68
Lmphocytes	49.45	51.82	51.86	48.76	2.87	0.91	0.90	0.35
Monocytes	1.39	0.36	0.36	1.06	0.68	0.81	0.81	0.21
Eosinophils	0.92	1.02	1.52	2.02	0.16	0.01	0.08	0.23
Neu/lym ratio	1.09	1.08	1.02	1.05	0.14	0.70	0.91	0.90
Gran/mono ratio	495.63	147.88	227.52	246.21	92.47	0.37	0.08	0.06
Hematocrit, %	17.80	17.79	19.10	18.85	0.17	0.01	0.46	0.49

Table 12. Effect of mixing and age at weaning on peripheral blood leukocyte concentrations and proportions of pigs on day 65 after weaning.

	Weaning age = 14		Weaning age = 21			P-value		
× 10^3/µL	Mixed	Unmixed	Mixed	Unmixed	SE	Age	Mixed	Interaction
WBC	6.23	5.40	5.11	3.75	0.73	0.07	0.14	0.71
Neutrophils	2.76	2.34	2.27	1.08	0.63	0.18	0.21	0.55
Lymphocytes	3.04	2.83	2.53	2.40	0.32	0.16	0.60	0.90
Monocytes	0.02	0.02	0.03	0.02	0.01	0.88	0.28	0.51
Eosinophils	0.15	0.15	0.18	0.12	0.03	0.99	0.33	0.38
% WBC								
Neutrophils	40.97	42.17	31.33	26.93	5.99	0.05	0.79	0.64
Lmphocytes	51.23	51.45	61.88	65.79	5.18	0.02	0.69	0.72
Monocytes	0.39	0.36	0.45	0.43	0.07	0.38	0.78	0.95
Eosinophils	2.44	2.97	3.30	3.10	0.58	0.40	0.78	0.53
Neu/lym ratio	0.93	0.91	1.07	0.72	0.21	0.90	0.40	0.43
Gran/mono ratio	172.29	171.50	180.24	89.08	56.35	0.49	0.40	0.41
Hematocrit, %	17.32	16.80	16.66	16.70	0.57	0.51	0.67	0.63

Table 13. Effect of age at weaning on macrophage phagocytic capacity, lymphocyte proliferation response, and interferon-γ production of pigs during the nursery phase.

	Weaning age			
Item	14 d	21 d	SE	P-value
Macrophage phagocytosis[a]				
Phagocytic macophages, %	7.16	10.71	1.76	0.173
SRBC consumed	1.40	1.51	0.11	0.491
Lymphocyte proliferation, cmp [b]				
Unstim	2,812	518	1,421	0.271
PHA,40 µg/mL	130,135	105,796	13,557	0.222
PWM, 15 µg/mL	40,413	28,915	4,628	0.100
ConA, 25 µg/mL	130,578	81,635	19,834	0.100
Interferon-γ[c] (SI)	896	828	178	0.792

[a] Macrophage phagocytic capacity was determined by evaluating the percentage of macrophages that were phagocytic (consumed at least one sheep red blood cell) and the average number of sheep red blood cells consumed by each phagocytic macrophage.

[b] Lymphocyte proliferation was measured as counts per minute (cpm) in unstimulated cell cultures and cultures stimulated with phytohemagglutinin (PHA), pokeweed mitogen (PWM), and concanavalin A (ConA) to induce proliferation of lymphocytes.

[c] Interferon-γ values are reported as a stimulation index (SI), calculated by subtracting the concentration of interferon-γ produced by cell cultures stimulated with concanavalin A from the concentration of interferon-γ produced by unstimulated cell cultures divided by the concentration of the unstimulated cultures.

Table 14. Effect of mixing and age at weaning on macrophage phagocytic capacity and lymphocyte proliferation response of pigs during the growing/finishing phase.

	Weaning age = 14		Weaning age = 21			P-value		
Trait	Mixed	Unmixed	Mixed	Unmixed	SE	Age	Mixed	AxM[a]
Macrophage phagocytosis[b]								
Phagocytic macrophages, %	9.24	8.87	11.27	10.81	2.24	0.381	0.853	0.985
SRBC consumed	1.51	1.44	1.49	1.47	0.09	0.938	0.636	0.800
Lymphocyte proliferation, cmp [c]								
Unstim	1,847	1,305	884	981	262	0.020	0.402	0.231
PHA,40 μg/mL	118,352	96,318	98,761	107,120	11,830	0.713	0.567	0.208
PWM, 15 μg/mL	40,588[y]	30,322[z]	29,760[z]	39,962[y]	3,445	0.864	0.993	0.006
ConA, 25 μg/mL	122,602	93,908	98,689	105,409	11,115	0.581	0.330	0.121
Interferon-γ[d] (SI)	1,382	1,249	1,289	1,330	255	0.980	0.858	0.735

[a] Age x mixed interaction.

[b]Macrophage phagocytic capacity was determined by evaluating the percentage of macrophages that were phagocytic (consumed at least one sheep red blood cell) and the average number of sheep red blood cells consumed by each phagocytic macrophage.

[c]Lymphocyte proliferation was measured as counts per minute (cpm) in unstimulated cell cultures and cultures stimulated with phytohemagglutinin (PHA), pokeweed mitogen (PWM), and concanavalin A (ConA) to induce proliferation of lymphocytes.

[d] Interferon-γ values are reported as a stimulation index (SI), calculated by subtracting the concentration of interferon-γ produced by cell cultures stimulated with ConA from the concentration of interferon-γ produced by unstimulated cell cultures divided by the concentration of the unstimulated cultures.

[y,z] Means with different superscripts are statistically different ($P < 0.05$).

Table 15. Average daily gain, average daily feed intake, and gain:feed of pigs in response to weaning age during the nursery period.

	Weaning Age, d			
Trait	14	21	SE	*P*-value
ADG, g				
Phase 1	262	383	15.3	<0.001
Phase 2	487	612	14.7	<0.001
Phase 1-2	397	521	13.9	<0.001
ADFI, g				
Phase 1	273	410	15.9	<0.001
Phase 2	617	876	29.1	<0.001
Phase 1-2	474	689	24.1	<0.001
Gain:feed				
Phase 1	0.92	0.94	0.01	0.095
Phase 2	0.77	0.70	0.01	0.001
Phase 1-2	0.81	0.76	0.01	0.001
Weight, kg				
Initial	4.52	6.67	0.26	<0.001
Phase 1	8.17	12.06	0.45	<0.001
Phase 2	18.39	24.92	0.72	<0.001

Table 16. Average daily gain, average daily feed intake, and gain:feed of pigs in response to weaning age during the growing/finishing period.

	Weaning Age, d			
Trait	14	21	SE	P-value
ADG, kg				
Phase 1	0.76	0.77	0.02	0.593
Phase 2	1.01	0.98	0.02	0.258
Phase 3	1.04	0.86	0.02	<0.001
Phase 4	0.88	0.86	0.04	0.719
Phase 1-4	0.91	0.87	0.01	0.005
ADFI, kg				
Phase 1	1.56	1.61	0.03	0.199
Phase 2	2.43	2.30	0.05	0.075
Phase 3	2.76	2.59	0.06	0.040
Phase 4	3.03	2.88	0.08	0.190
Phase 1-4	2.26	2.30	0.04	0.509
Gain:feed				
Phase 1	0.49	0.48	0.01	0.396
Phase 2	0.42	0.43	0.01	0.360
Phase 3	0.38	0.33	0.01	0.003
Phase 4	0.29	0.30	0.01	0.757
Phase 1-4	0.40	0.38	0.01	0.003

Table 17. Average daily gain, average daily feed intake, and gain:feed of pigs that were mixed and resorted at the initiation of the finishing period or remained unmixed during the finishing period.

	Finishing Treatment			
Trait	Mixed	Unmixed	SE	P-value
ADG, kg				
Phase 1	0.76	0.77	0.02	0.598
Phase 2	1.02	0.98	0.02	0.242
Phase 3	0.92	0.97	0.02	0.145
Phase 4	0.86	0.88	0.04	0.671
Phase 1-4	0.88	0.89	0.01	0.607
ADFI, kg				
Phase 1	1.59	1.58	0.03	0.691
Phase 2	2.41	2.31	0.05	0.167
Phase 3	2.64	2.71	0.06	0.345
Phase 4	2.91	3.01	0.08	0.357
Phase 1-4	2.28	2.29	0.04	0.831
Gain:feed				
Phase 1	0.48	0.49	0.01	0.198
Phase 2	0.42	0.42	0.01	0.864
Phase 3	0.35	0.36	0.01	0.568
Phase 4	0.30	0.30	0.01	0.887
Phase 1-4	0.39	0.39	0.01	0.924

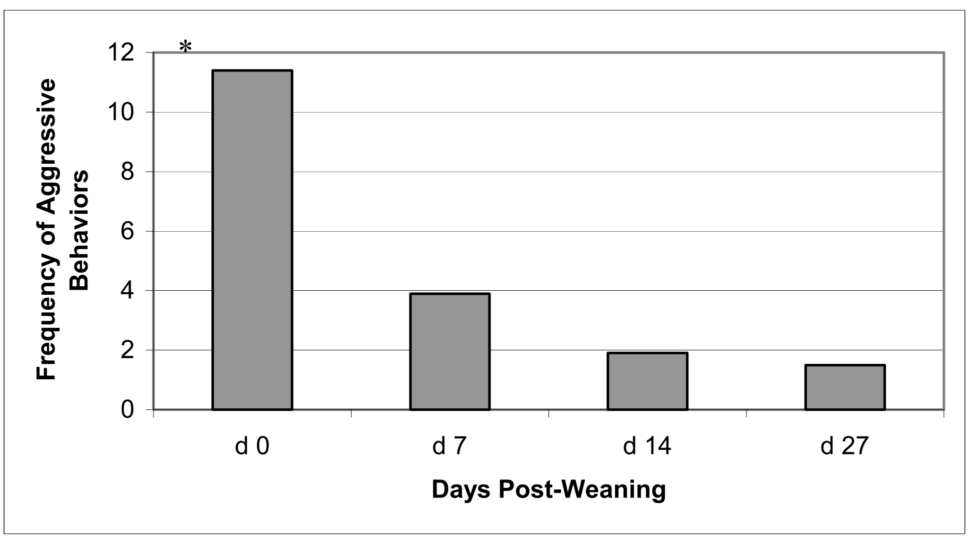

Figure 1. Frequency of aggressive behaviors observed on each day of the nursery phase. Bars with an asterisk differ from other bars without asterisks ($P < 0.05$).

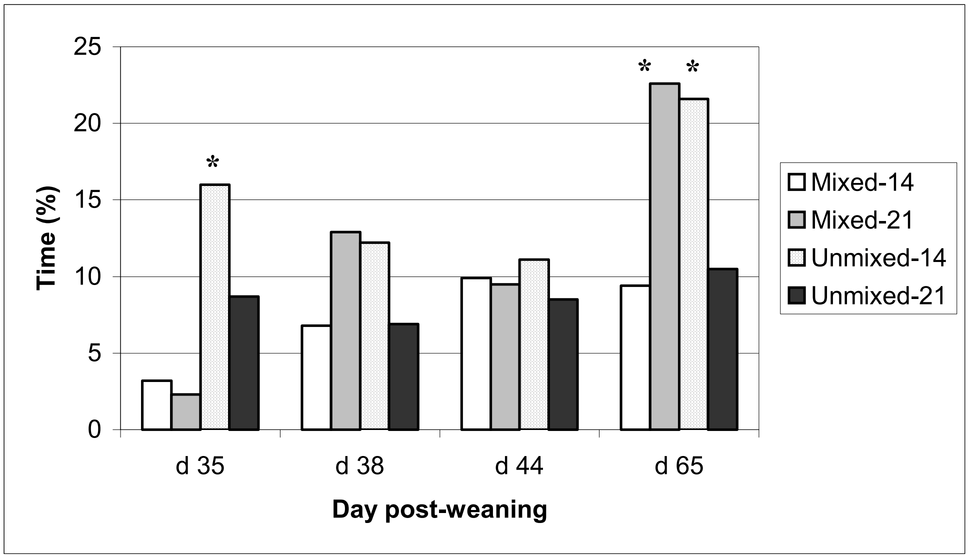

Figure 2. Percentage of time spent engaged in feeding behavior during the growing/finishing phase by pigs weaned at either 14 or 21 d of age and either subjected to mixing and resorting following the nursery phase or remaining in original pens (weaning age x mixing x date interaction, $P < 0.05$). Bars with an asterisk differ from other bars without asterisks within day post-weaning ($P < 0.05$).

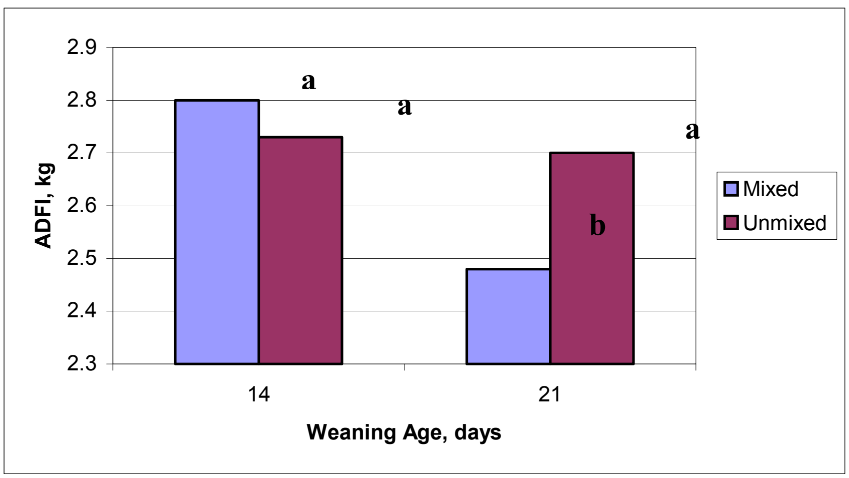

Figure 3. Effect of mixing and age at weaning on average daily feed intake of pigs (ADFI) during Phase 3 of the growing/finishing period (interaction, $P = 0.08$; a,b $P < 0.05$).

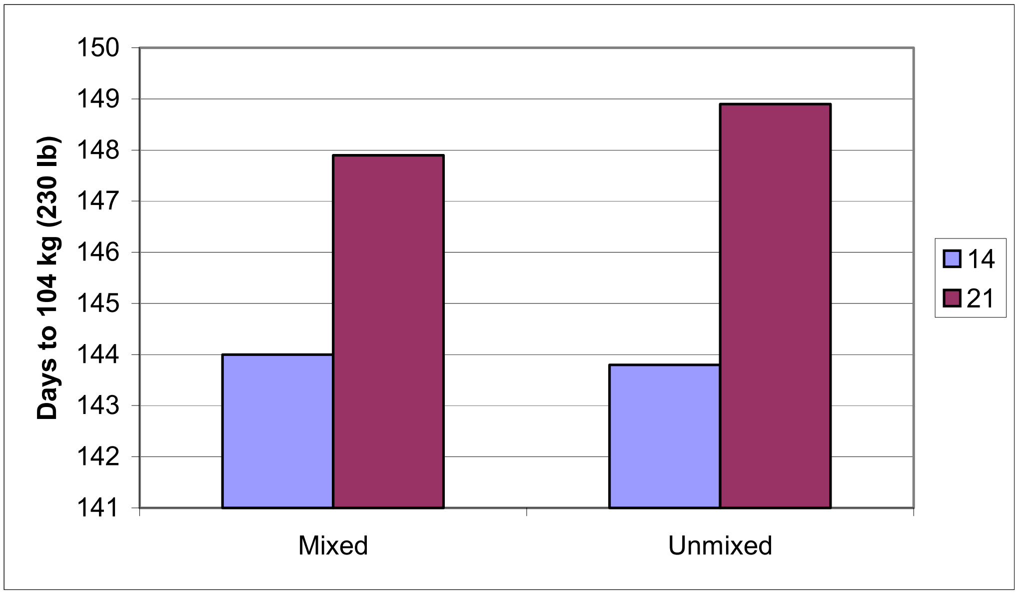

Figure 4. Effect of mixing and age at weaning on the number of days for pigs to reach 230 lb. Effect of weaning age; $P < 0.05$.