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Effect of vaccination against *Mycoplasma hyopneumoniae* on health, growth, and pubertal status of gilts exposed to moderate ammonia concentrations in all-in–all-out versus continuous-flow systems

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Summary

Objective: To determine whether vaccination against *Mycoplasma hyopneumoniae* during exposure to ammonia alters prevalence and severity of lung lesions, lean tissue weight, and onset of puberty in gilts reared in all-in–all-out (AIAO) or continuous-flow (CF) production facilities.

Methods: Two hundred and sixteen Yorkshire × Hampshire × Chester White gilts were reared in either AIAO or CF facilities at the Purdue University Animal Research Center during exposure to ammonia from 2.5–6 months of age. Half of the gilts in each environment were vaccinated against *Mycoplasma hyopneumoniae*. Lean tissue weight, prevalence and severity of lung lesions, and onset of puberty in gilts were determined at slaughter. Sera titers of *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* were measured as gilts entered and exited the study. Correlations of sera titers with severity of lung lesions and with days-to-104.5 kg (230.4 lb) were calculated.

Results: Average daily gain was greater ($P < .001$) for gilts raised in AIAO versus CF facilities, but was not significantly greater for vaccinates than nonvaccinates. Feed:gain ratios were similar among AIAO and CF gilts, with or without vaccination. Gilts reared in AIAO facilities tended ($P = .08$) to reach 104.5 kg (230.4 lb) bodyweight (BW) in fewer days than those raised in CF facilities (172 versus 180 days) and than those gilts that were not vaccinated (173 versus 179 days; [$P = .07$]). Fewer lung lesions were present in AIAO gilts than in CF gilts ($P < .001$). Vaccination did not reduce ($P = .15$) prevalence of lesions in either environment. Antibody titer of *M. hyopneumoniae* and *A. pleuropneumoniae* were positively correlated ($P < .05$) with severity of lung lesions at slaughter but no relationship ($P > .05$) was found between antibody titer and days-to-104.5 kg (230.4 lb) BW. Neither animal flow strategy (AIAO versus CF) nor vaccination affected ($P > .05$) ovarian weight, uterine weight or length, or percentage of gilts cycling after exposure to boars.

Implications: Lung lesions detected at slaughter were less numerous and growth of gilts was improved by using AIAO facilities rather than CF facilities. Vaccination of gilts against *Mycoplasma hyopneumoniae* did not produce significant benefits.

Keywords: swine, gilts, all-in–all-out (AIAO), lean weight, lung lesions, mycoplasmal pneumonia, puberty

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Enzootic pneumonia associated with *Mycoplasma hyopneumoniae* and *Pasteurella multocida* is the most costly respiratory disease in the swine industry.[1] Depending upon the prevalence and severity of the infection, this disease can compromise feed consumption and weight gain, resulting in reduced growth performance.[1] Previous studies have shown that vaccination against *M. hyopneumoniae* reduces the severity of lung lesions detected at slaughter and improves feed efficiency and average daily weight gain (ADG) during the finishing phase.[2]

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Using all-in–all-out (AIAO) strategies to rear nursery and grow-finish pigs has improved rate of gain and shortened days to market.[3] All-in–all-out interrupts the cycle of pathogen transmissions from older pigs to younger pigs, and allows the producer to tailor environmental conditions to a uniform population of pigs and to clean the facilities between groups of pigs. The economic benefits of converting or remodeling buildings from conventional continuous-flow (CF) design to AIAO production have been estimated at $1.31 per hog sold.[4]

Growing gilts exposed to moderate aerial concentrations of ammonia (35 ppm) have been observed to have reduced growth performance.[5] The objectives of the study reported here were to determine whether health, reproductive, and lean growth performance were improved in gilts reared in a moderate aerial ammonia atmosphere in AIAO versus CF facilities and vaccinated against *M. hyopneumoniae*.

Materials and methods

All gilts that participated in the study were obtained from a herd free from pseudorabies virus (PRV), swine influenza virus (SIV), and transmissible gastroenteritis virus (TGEV), but seropositive for subclinical porcine reproductive and respiratory syndrome virus (PRRSV). Previous slaughter checks from this herd indicated that the pigs were positive for *Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae,* and *Pasteurella multocida*.

Experimental design

Vaccination

Thirty-six gilts (Yorkshire × Hampshire × Chester White) were weaned at 28 days of age (week 0 of the study) into a nursery facility at Purdue University's Animal Sciences Swine Research Center (Figure 1). In the first replication, half of the gilts were randomly assigned to VAC treatment and received an IM injection of 2 mL of *M. hyopneumoniae* vaccine (RespiSure®, Pfizer AHG, Exton, Pennsylvania) in the neck at 1 and 4 weeks of age. The other half were designated NONVAC gilts and received no injection.

There were three additional replications of this study. Additional gilts were weaned monthly into the nursery and randomly allocated to one of the vaccination treatments so that over the course of the entire study (four replications), the number of VAC gilts equaled the number of NONVAC gilts.

AIAO versus CF

When they reached approximately 2.5 months of age (week 8 for the first replication), gilts in the nursery were injected with ivermectin (Ivomec®, Merck and Co., Rahway, New Jersey) and moved to one of two grow/finish rooms. One room was designated the AIAO room, and received 27 randomly selected gilts (15 VAC gilts and 12 NONVAC) (nine pens with three gilts per pen) (Figure 1). The other room was designated the CF room, and (in the first replication) received three VAC gilts and six NONVAC gilts. One month later (week 13 of the study), nine 2.5-month-old gilts from the nursery (six VAC and three NONVAC) were moved to the CF room. In week 17, nine more 2.5-month-old gilts (three VAC and six NONVAC) were added from the nursery to fill the pens in the CF room (Figure 1).

In replications two and four, 12 AIAO gilts and 15 CF gilts received the vaccine, while replication three repeated replication one, so that over the four total replications there were 108 gilts in each vaccination treatment, sorted by weight .

The VAC and NONVAC gilts were alternated among adjacent pens in both rooms to form the following grow-finish treatment groups:

- AIAO VAC (n = 54);
- AIAO NONVAC (n =54);
- CF VAC (n = 54); and
- CF NONVAC (n = 54).

Seeder pigs

For the first replication only, nine seeder pigs (4–5 months of age) were moved into the CF room into three pens adjacent to the CF gilts to allow nose-to-nose contact (Figure 1). At week 17, the seeder pigs were removed from the CF room and their pens were filled with the third group of nine 2.5-month-old study gilts. The seeder pigs were obtained from a herd with a herd history of *M. hyopneumoniae*, *P. multocida,* and *A. pleuropneumoniae.* For replications two through four, the older gilts already in the CF room served as seeder pigs to transmit the pathogens to the newly added gilts.

Slaughter

At the end of the grow/finish period (week 27), all gilts were removed from the AIAO room over a 2-day period (due to abattoir space limitations). The AIAO room was then disinfected with a power washer and repopulated with the next replication, until all four replications had been completed. Also in week 27, the nine oldest CF gilts were sent to slaughter and replaced with the first nine gilts of the second replication.

Facilities

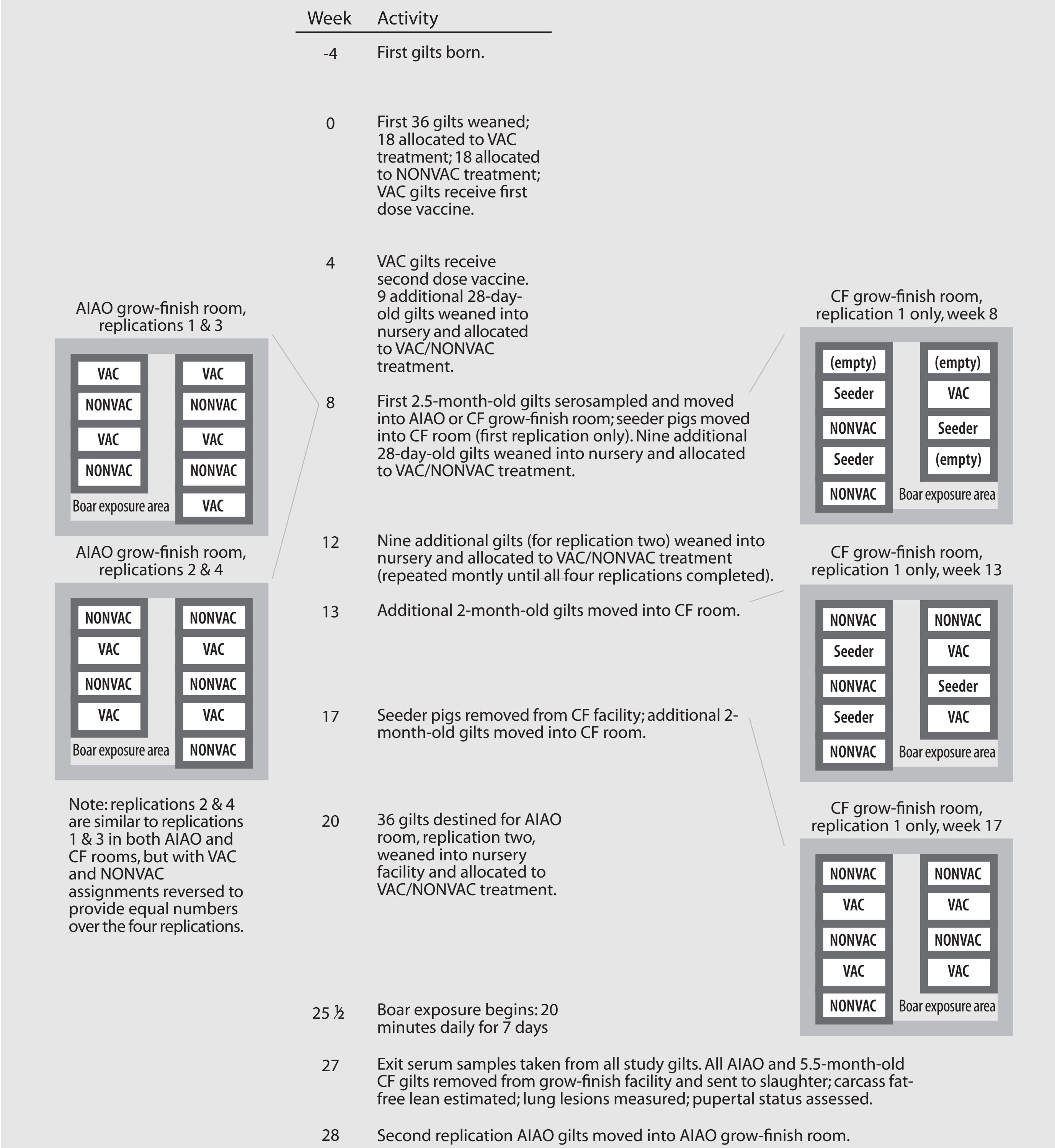
The grow-finish rooms used in this study were environmentally controlled rooms at the Purdue University Animal Sciences Swine Research Center. Each room had a woven-wire floor and contained nine pens with 3 pigs per pen (1.2 × 2.0 m to allow 0.80 m^2 per gilt), each with a two-hole feeder and one-nipple waterer. Each of the environmentally regulated rooms had independently controlled, identical ventilation systems and layout. A ventilation rate of 33.6 m^3 per minute in each room was maintained. Temperature in each room during the experiment was maintained at 25°C (77°F). The two rooms had separate but identical shallow-pit manure storage systems.

Ammonia

To maintain moderate concentrations of ammonia in both rooms, manure and urine was allowed to accumulate in the pit with an automatic overflow set at the 0.48-m depth. Aerial ammonia concentrations were obtained initially and maintained by adding anhydrous ammonia from a steel tank; a regulator and asparger tube were submerged beneath the liquid manure in one of the central channels. The asparger tube had 0.3-cm holes spaced at 10-cm intervals to evenly distribute the ammonia into manure and, subsequently, into the atmosphere. After several weeks of manure accumulation in the pit, little ammonia was added from the tank because sufficient ammonia was released naturally from manure decomposition.

Air samples were obtained weekly in each room using a volumetric pump (Sensidyne/Gastec, Largo, Florida). Ammonia concentration was determined with direct-read color-detector tubes with a range of 1–60 ppm. Replicate air samples were obtained at random locations 0.3 m (1 foot) above the floor in each room to determine mean concentration. When ammonia was added to the manure pit, aerial ammonia concentration was also measured after ammonia discharge.

Figure 1



Study design and timeline

Growth

Gilts were weighed every 2 weeks throughout the experiment in their respective rooms. Feeders were also weighed back at 2-week intervals to calculate feed efficiency. When the gilts averaged 68 and 89 kg (150 and 196 lb) bodyweight (BW), fat-free estimates of lean tissues were obtained by bioelectrical impedance (Model BIA-101, RJL Systems, Detroit, Michigan) and ultrasound (Aloka Corometrics 210 DX Real-Time Ultrasound Scanner, Corometrics Medical Systems, Inc., Peachtree City, Georgia) using prediction equations developed from previous trials.[6] At slaughter (approximately 112 kg [246 lb]), estimates of carcass fat-free lean were determined by total body electrical conductivity (TOBEC; MQ-25 Electromagnetic Scanner, Meat Quality Inc., Springfield, Illinois).[6,7]

Serology

Blood samples were drawn by venipuncture when gilts entered the grow-finish rooms, and a second blood sample was obtained at slaughter. Samples were placed on ice at collection. Sera were harvested by centrifugation at 1560 × g for 30 minutes and stored at -20°C until analyzed for presence of antibodies to *M. hyopneumoniae* using the Tween 20 ELISA (Bayer Veterinary Labs, Inc., Worthington, Minnesota) and for antibodies to serotypes 1, 5, 9, 10, and 11 of *A. pleuropneumoniae* using the hemolysin-inhibition method.[8]

For classification for *A. pleuropneumoniae,* samples with titers:

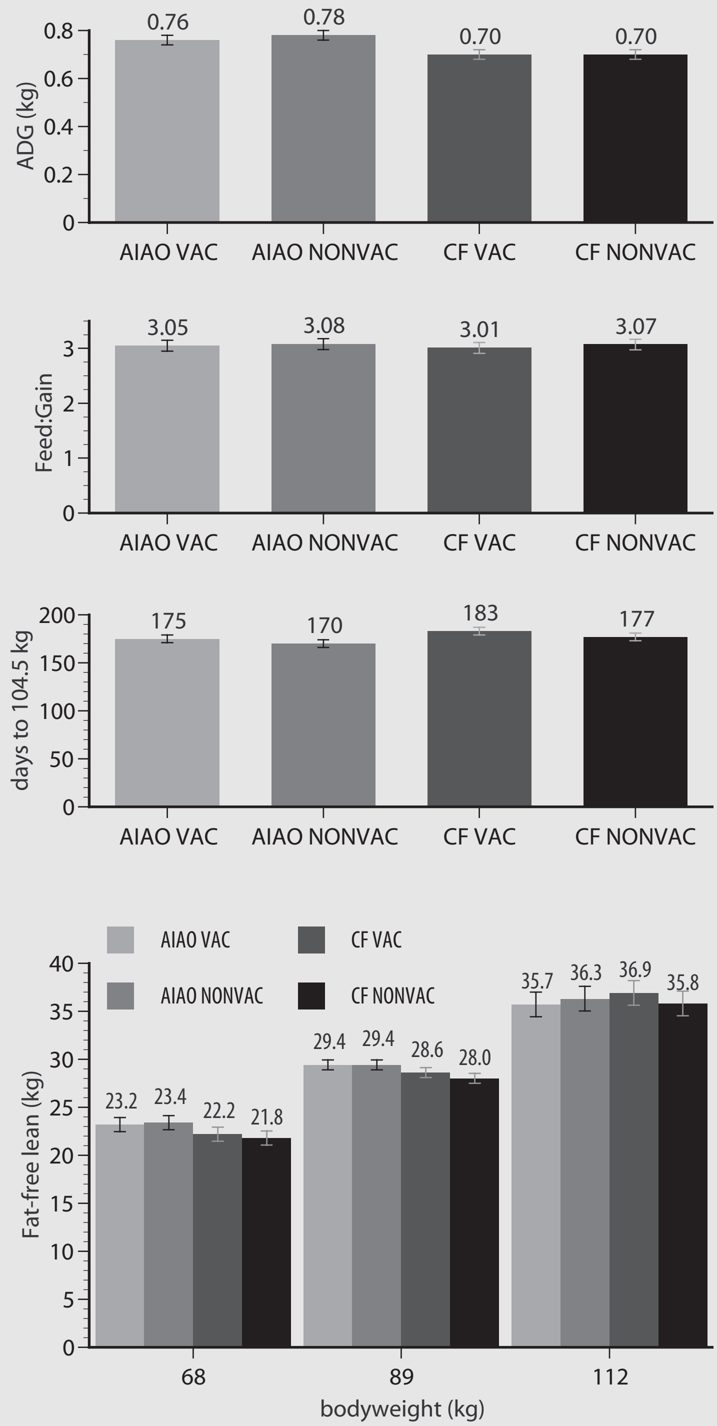
- less than 3000 were interpreted to be negative (score = 1),
- between 3000 and 6000 were interpreted to be suspect (score = 2), and
- greater than 6000 were interpreted to be positive (score = 3).

Strains of *Actinobacillus suis* produce an immunologically similar toxin that elicits an antibody that cross-reacts to *A. pleuropneumoniae* in this assay. A change in titer score for each gilt was obtained by comparing initial and slaughter titers and tabulating the difference as Δ + 3.

Serological results for antibody titers to *M. hyopneumoniae* were based on the optical density results from the ELISA and categorized as negative, suspect, or positive. For classifications, samples that had optical densities:

- less than the mean ± 2 standard deviations (SD) from the negative control sample were interpreted to be negative (score = 1),
- greater than the mean ± 2 SD were interpreted to be suspect (score = 2), and
- 10% above the suspect cutoff were interpreted to be positive (score = 3).

Figure 2



Growth data from study gilts

A change in titer score for each gilt was obtained as described above.

Pubertal status

At approximately 6 months of age and 106 ± 1 kg (233.7 ± 2.2 lb) BW, gilts were exposed to mature boars for 20 minutes daily for 7 days. Four boars were housed in a separate facility and were rotated between the rooms to minimize the effects of ammonia on the boars. Three to 4 days after the final boar exposure, gilts were slaughtered and reproductive tracts were recovered to determine pubertal status. For each gilt, the adrenal gland, ovaries, and uterus were weighed, the length of the uterus was measured, and the number of follicles present were counted.

Measurement of lung lesions

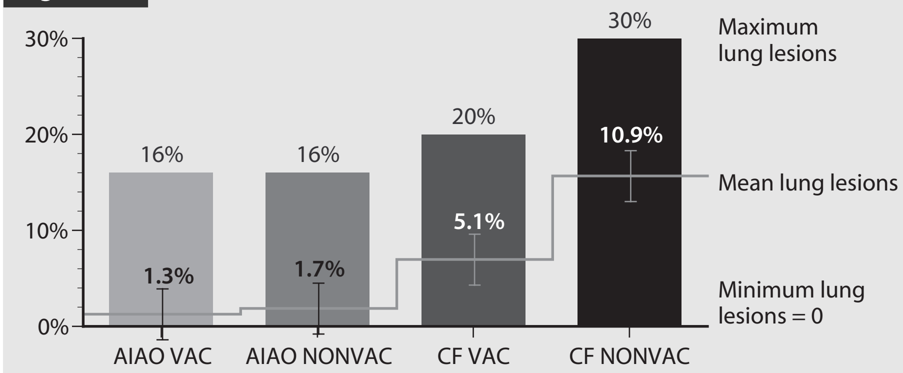
At slaughter, dorsal surfaces of the cranial, middle, and caudal lobes of both lungs and the ventral surface of the accessory lobe were examined for gross lesions consistent with enzootic pneumonia.[9] Sketches of all lesions were made on a diagram of the lungs. Percentage of area of lesions in each diagram was determined, using a computerized digitizer (Osteoplan, Kontron Image Analysis Division, Zeiss Co., New York, New York).

Statistical analyses

Means for health and lean tissues measures of the four treatment groups were compared by subjecting data to least-squares ANOVA, using general linear model procedures from Statistical Analytical Systems (SAS).[10] Replication (REP) was a random effect and environment (ENV; AIAO versus CF) and vaccination (VAC) were fixed effects in a 2×2 factorial arrangement of treatments. REP × ENV and REP × VAC were used as the error terms to test significance of ENV and VAC effects, respectively.

Prevalence of gilts exhibiting lung lesions were analyzed by χ^2.[11] Pearson's correlation coefficients were calculated and used to establish relationships among severity of lung lesions, days-to-104.5 kg (230.4 lb), and *M. hyopneumoniae* and *A. pleuropneumoniae* antibody classification titers in sera.[10] "Suspect" and "positive" serology results were pooled for this analysis. Data from four gilts in the CF-VAC and four gilts in the CF-NONVAC treatment groups were excluded from statistical analyses due to lameness or death.

Figure 3



Lung lesion data from study gilts

Results

Ammonia

Aerial concentration of ammonia was maintained at a mean concentration of 23 ppm for the study, and did not differ between rooms ($P > .05$) for each environment across all four replications of the experiment.

Growth

There were no significant ($P > .05$) interactions between environment (i.e., AIAO versus CF) and vaccination treatments. Average daily gain was greater ($P < .001$) in AIAO than CF facilities (Figure 2), but was not influenced by vaccination ($P = .43$). Feed:gain ratio did not differ ($P > .05$) between either VAC/NONVAC or between AIAO versus CF gilts. The number of days to reach 104.5 kg (230.4 lb) BW tended ($P < .08$) to be less for those gilts reared in AIAO facilities versus CF gilts and for NONVAC gilts than for VAC gilts. At slaughter, AIAO gilts were heavier ($P = .003$) than CF gilts. Neither environment nor vaccination produced significant effects ($P > .26$) on carcass lean measurements.

Lung lesions

Percentage of lung affected by lesions was less ($P < .001$) in AIAO than CF gilts (Figure 3). Each treatment group contained gilts without detectable lesions, but the prevalence of lesions was higher ($P < .005$) in the CF gilts than in the AIAO gilts. Vaccination against mycoplasmal pneumonia did not reduce ($P = .15$) prevalence of lesions in either environment.

Pubertal status

Neither environment nor vaccination was associated with a difference ($P > .05$) in adrenal weight, ovarian weight, uterine weight or length, or the percentage of gilts cycling as determined by the presence of corpora lutea 10 days after exposure to boars (Figure 4). The total number of follicles 4 mm or larger in diameter were greater ($P < .05$) in the AIAO gilts than in the CF gilts, but vaccination had no effect ($P = .88$) on follicle numbers.

Serology

Comparison of entry and exit titers suggests that *M. hyopneumoniae* and *A. pleuropneumoniae* were transmitted to CF gilts but not AIAO gilts (Figure 5). Sera titers of *M. hyopneumoniae* and *A. pleuropneumoniae* were positively correlated ($P < .05$) with severity of lung lesions, but no relationship ($P > .05$) was found between sera titers and days-to-104.5 kg (230.4 lb) BW (Table 1).

Discussion

Our observation that ADG was improved in AIAO gilts compared to CF gilts is consistent with earlier studies[3,12] in which AIAO pigs gained approximately 10% faster than CF pigs. Feed efficiency improvement in AIAO facilities has varied between 1%–6% among experiments.[3] However, the 7-day reduction of days to market (104.5 kg, 230 lb) we

Table 1

Simple correlations in study gilts

	Days to 104.5 kg bodyweight	*M. hyopneumoniae*	*A. pleuropneumoniae*
Severity of lung lesions	.040	.179*	.184*
Days to 104.5 kg bodyweight		.052	.087
Mycoplasma hyopneumoniae			-.047

* $P < .05$

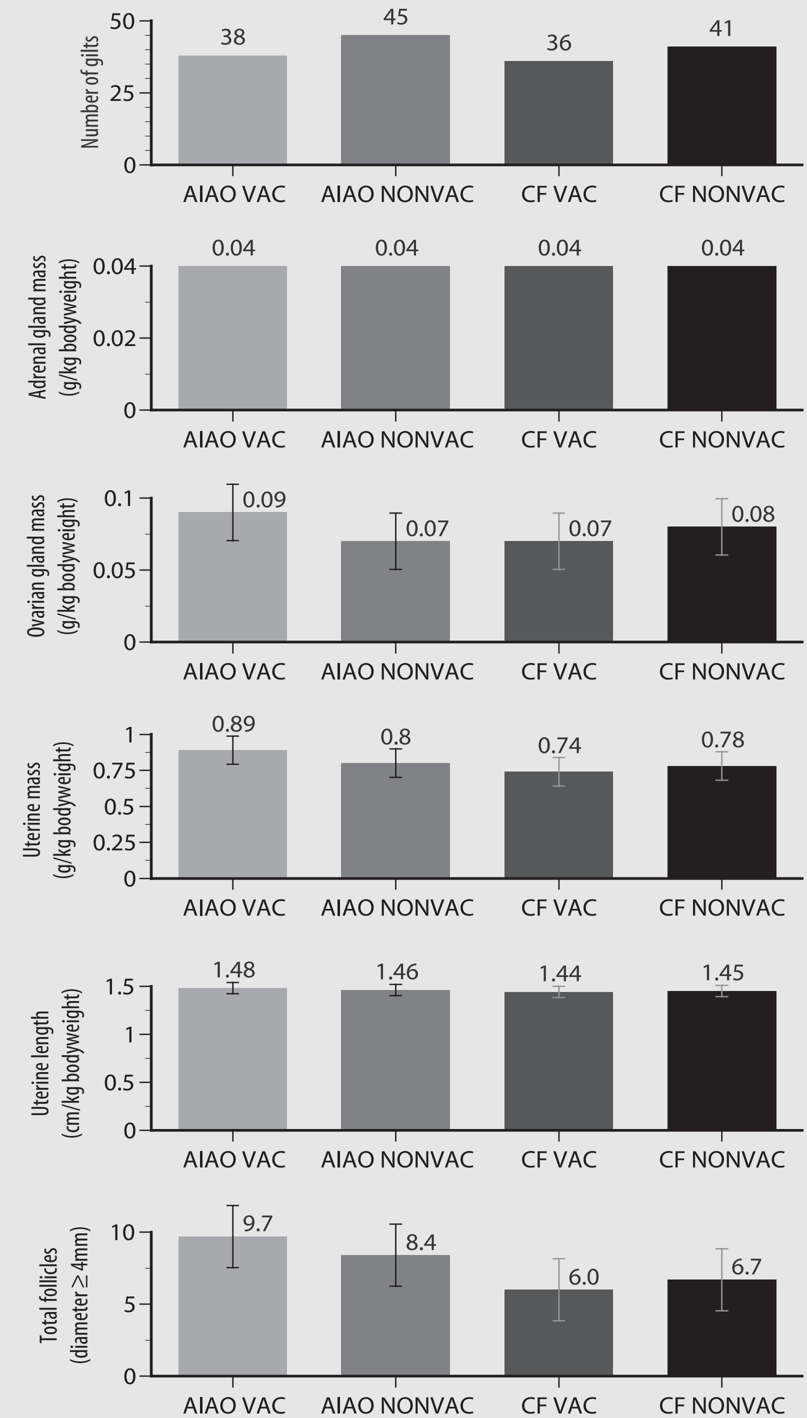
observed was not as robust as reported previously.[3] This discrepancy is likely due to different facility and management conditions and different herd genetics. However, further research is needed to clarify the relationship between animal flow strategies and days to market.

Exposure of gilts to moderate aerial concentrations of ammonia has been observed to dampen growth performance in a previous study.[5] The experimental design of the present study did not allow us to compare growth performance in AIAO versus CF environments under elevated ammonia concentrations to that of AIAO versus CF environments under low ammonia concentrations. Further research is required to clarify the relationship of ammonia concentrations and growth under AIAO versus CF conditions.

Our observation that the performance of vaccinates was not improved over nonvaccinates is inconsistent with an earlier study in which pigs vaccinated for mycoplasmal pneumonia in a commercial herd affected with enzootic pneumonia gained 7.7% faster.[13] Rearing vaccinates and nonvaccinates in the same air space may have contributed to our results. Concurrent infection with several serotypes of *A. pleuropneumoniae* may also have confounded the result.

Depressed growth rate has been associated with pneumonia in several studies.[14–16] In earlier experiments, it was uncertain when the relationship between *M. hyopneumoniae* and presence of lung lesions was established and how it ultimately affected growth parameters.[17] Our observation that growth was not correlated with antibody titers to *M. hyopneumoniae* or severity of lung lesions does not establish a cause and effect relationship. Further research, using the current design but including serial slaughtering and/or serologic testing at monthly intervals, is needed to clarify the progression of pneumonic lesion development.

Figure 4

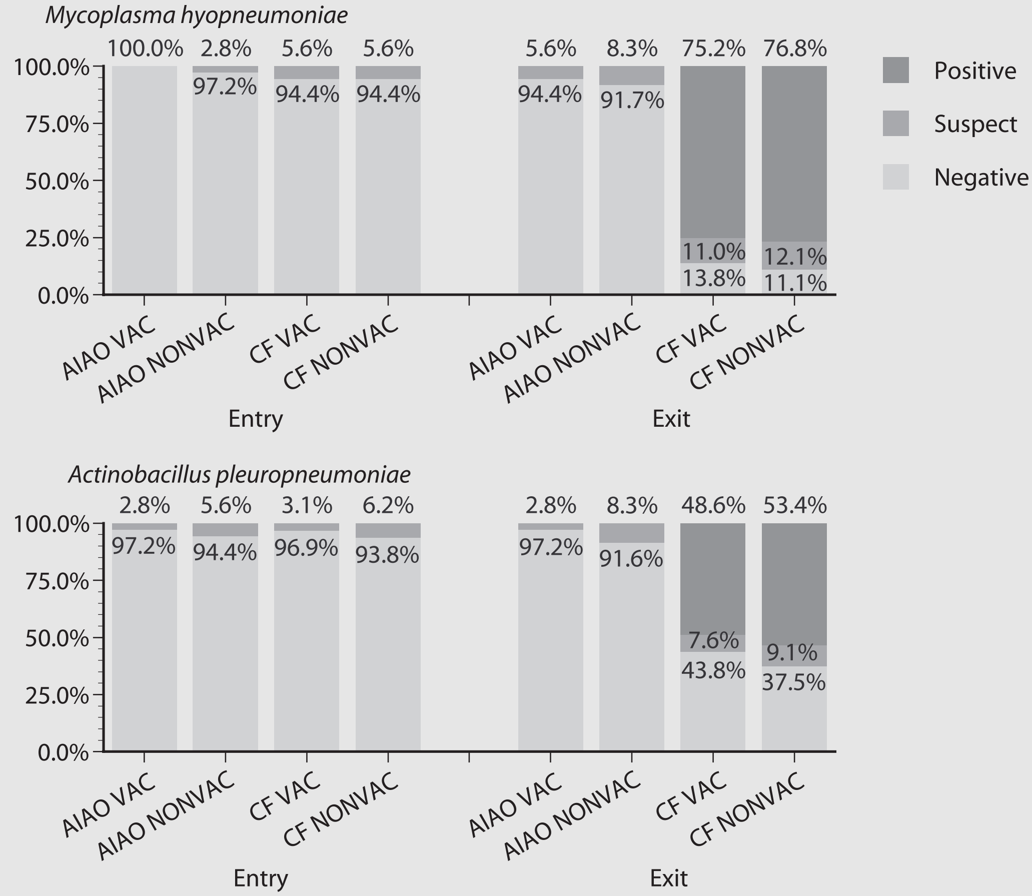


Pubertal status of study gilts

Implications

- Average daily gain was greater for gilts raised in AIAO versus CF facilities, but was not different between vaccinated and nonvaccinated gilts.
- Fewer lung lesions were present in gilts reared in AIAO than CF facilities, but vaccination did not reduce prevalence of lesions in either environment.
- Antibody titer of *M. hyopneumoniae* and *A. pleuropneumoniae* were positively correlated with severity of lung lesions at slaughter, but no relationship was found between antibody titer and days-to-104.5 kg (230.4 lb) body weight.

Figure 5



Serology status of study gilts for *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*

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