

# A possible relationship between low facility dust and endotoxin levels and improved growth rates in pigs reared by Isowean<sup>SM</sup>

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## Summary

**Purpose:** To explore the association of the biological difference (i.e., growth rates and thymus gland weights) between pigs weaned in isolation from their dams versus those reared in conventional nurseries.

**Methods:** Dust concentrations and total and respirable endotoxin were measured in two types of swine-rearing facilities: an isolated nursery and nursery within a conventional farm.

**Results:** We found isolated nurseries in this study had significantly ( $P < .05$ ) less dust, and total and respirable endotoxin and pigs had greater growth rates.

**Implications:** There may be an association between environmental facility contaminants and growth rate.

**Keywords:** swine, dust, endotoxins, growth performance

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Isolated weaning (Isowean<sup>SM</sup>) is a method by which sows farrow in the conventional herd and piglets are weaned at 5–21 days of age and moved to a nursery physically located away from the farrowing site. Weaning age, medication, and approach depends on the disease to be eliminated.<sup>1</sup> The exact mechanisms that eliminate disease have not been determined. Possible reasons include colostral immunity, antibiotics before removing pigs from the source herd, and/or age of weaning.<sup>2</sup>

Isowean<sup>SM</sup> has been successful in eliminating *Mycoplasma hyopneumoniae*, toxigenic *Pasteurella multocida*, transmissible gastroenteritis virus (TGEV), and pseudorabies virus (PRV).<sup>1,3–6</sup> Studies have observed that pigs also have improved performance when compared to their counterparts that remain in the herd of origin.<sup>7,8</sup> Harris, et al., reported 30% improved pig performance in pigs raised in an isolated nursery compared with the control pigs that remained in the herd of origin.<sup>7</sup>

Environmental contaminants present in swine confinement buildings

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may have detrimental effects on both humans and animals. The purpose of this study was to measure and compare environmental contaminants in an isolated nursery and a nursery within a conventional farm. Specific objectives included:

- to determine concentrations of total and respirable endotoxin from dust samples;
- to determine whether significant differences exist between contamination in isolated facilities versus conventional facilities; and
- to evaluate biological performance in the two systems.

## Materials and methods

Three trials were conducted in which dust and total and respirable endotoxin were measured. Each trial used the same conventional farm, began with pigs 15–17 days of age, and continued for 8 weeks. Environmental samples were collected from 3:00–6:00 for Trials 1 and 3, and from 21:00–24:00 for Trial 2 because these were periods of decreased human and animal activity. Trials were conducted in winter (Trial 1: December 1991–January 1992), spring (Trial 2: March–April 1992), and fall (Trial 3: August–October 1992).

Ninety-six pigs were used in Trials 1 and 3. Pigs were allocated into one of two treatment groups:

- isolated nursery (n=72) (treatment); or
- conventional nursery on the source farm (n=72) (control);

with two replicates per treatment (n=24 for each replicate).

Forty-eight pigs were used in Trial 2, in which pigs were allocated into one of two treatments:

- isolated nursery (n=24) (treatment); or
- conventional nursery on the source farm (n=24) (control).

## Conventional farm

The pigs' herd of origin studied in the three trials was a 500-sow farm located in south-central Kentucky approximately 1.6 km (1 mile) from any other pig farm. The facility, built in the early 1980s, consists of a breeding/gestation/farrowing barn, a two-stage nursery, a grower barn, and a finisher barn. It has shower-in/shower-out facilities and an office. The various barns are connected by a common enclosed hallway.

Each nursery room was individually ventilated; however, air was exchanged between second-stage nursery rooms via a common manure pit. Rooms were ventilated by a negative air pressure system.

The first-stage nursery consisted of three rooms with 16 pens. Each first-stage nursery room had a separate shallow manure pit. Each pen is 1.2 m × 1.2 m (4 ft × 4 ft), had mesh wire flooring over a shallow manure pit, and held eight to ten pigs for 16–21 days (end weight of 4–6 kg [9–13 lb]). The second-stage nursery consisted of four rooms with eight pens. The second-stage nursery rooms shared a common shallow manure pit. Each pen was 1.2 m × 2.7 m (4 ft × 9 ft), had mesh wire flooring over a shallow manure pit, and held 16–18 pigs for 16–21 days (6–20 kg [13–44 lb]). The shallow manure pits were drained (by pulling a plug) when the room was emptied. Any remaining manure was not cleaned out and was present when new pigs filled the room. Animals were fed a restricted diet for the first 2 weeks of age, then fed ad libitum. No bedding was used in either the first- or second-stage nursery.

When animals were removed, each area was sprayed with a detergent and allowed to soak for 30–60 minutes. These areas were then pressure washed with cold water at 1500 psi and then disinfected using a quaternary ammonia (PFD-6, BioSentry, Inc., Stone Mountain, Georgia) at the rate of 30 mL (1 oz) disinfectant per 3.8 L (2 gal) water.

## Isolated nursery

The isolated nursery used in the three trials was located in south-central Kentucky. It was 49.9 km (31 miles) away from the conventional source farm and approximately 1.6 km (1 mile) away from any other pig farm. The isolated nursery consisted of shower-in/shower-out facilities, an office, a necropsy area, and three nursery rooms. Each room was individually ventilated with automatic fans, i.e., no air was shared between rooms. Each room contained a separate shallow manure pit, which was flushed two times a week at the beginning of a trial and three times a week as the animals grew. Pen sizes were adjusted to duplicate the pig space allotment at the conventional farm (approximately 0.6 m<sup>2</sup> [6.5 sq. ft] per pig). Pigs were reared in raised decks with mesh wire floors for the duration of the trial. No bedding was used. Pigs were fed ad libitum for the duration of the study.

## Management

Animal caretakers for the isolated nursery and the nursery on the conventional farm did not come into contact with other pigs or sources of pathogen contamination and maintained strict standards of hygiene (shower-in–shower-out, entry only of supplies/equipment that had not been in contact with pigs and other sources of pathogen contamination). Personnel at the conventional farm did come into contact with pigs of various ages contained within the farm. Other personnel were restricted from entering the units until a 24-hour downtime period was observed.

## Dust measurement

Total dust samples were collected using personal air pumps (Gilian Instrument Corp., Wayne, New Jersey) calibrated to a flow rate of 1.7 L

(0.45 gal) per minute. The sampling interval was 3 hours. Respirable dust samples were collected using a cyclone preseparator (Mine Safety Appliance, Pittsburgh, Pennsylvania). Flow rates were set with a calibration rotometer (SKC, Eighty Four, Pennsylvania). Control samples were collected by removing end plugs from the filter cassette.

Dust samples were collected weekly (beginning at approximately 3 weeks of age [week 1 of trial]) for weeks 3–7 for Trial 1, weeks 1–5 for Trial 2, and weeks 1–7 for Trial 3. Each sample was collected in duplicate, plus one control filter. Blank filters were prepared in the same manner as dust filters. Blank filters were placed next to dust filters during the collection period. Dust samples were collected in adjacent empty pens at animal level.

Filters for total, respirable, and control dust samples were prepared in the following manner: polyvinyl chloride (PVC) filters (37 mm diameter, 0.5 µm pore size) (Omega Industries, Chelmsford, Massachusetts) were placed onto labeled support pads (Omega Industries, Chelmsford, Massachusetts). The filters and support pads were then placed into a vacuum desiccator (Nalgene, Rochester, New York) containing desiccation rocks (Drierite, Xenia, Ohio). A vacuum was pulled for 15 minutes. Weights were then determined using a microbalance (Cahn Instrument Inc., Cerritos, California). Once weights were recorded, the filter and support pad were placed into the filter cassette (Omega Industries, Chelmsford, Massachusetts). Cassettes were sealed with a gel band (SKC, Eighty Four, Pennsylvania) to prevent air leakage. Once samples were collected, the filter and support pad were removed from the filter cassette and placed in the vacuum desiccator for 15 minutes. Postweights were determined using the microbalance.<sup>9</sup>

The following equation was used to calculate mg dust per m<sup>3</sup> air:

$$\frac{(\text{postweight} - \text{preweight})_{\text{sample}} + (\text{postweight} - \text{postweight})_{\text{blank}}}{\frac{1}{1000} \times 180 \text{ minutes} \times \frac{1.7 \text{ L}}{1 \text{ minute}}}$$

## Endotoxin

Samples tested for endotoxin were collected from the dust samples by placing the postweighed filter into a 15-mL pyrogen-free centrifuge tube (Fisher Scientific, Stone Mountain, Georgia) filled with 10 mL of pyrogen-free water (BioWhittaker, Walkersville, Maryland).

A chromogenic assay (BioWhittaker, Walkersville, Maryland) was performed on the endotoxin samples. This assay quantified the amount of Gram-negative bacterial endotoxins in a solution with a modified *Limulus polyphemus* (horseshoe crab) amebocyte lysate and synthetic color-producing substrate.

Fifty µL of each sample, pyrogen-free water (blank), and four known endotoxin standards (1.0, 0.5, 0.25, and 0.1 endotoxin units (EU) per mL) were placed into pyrogen-free microtiter plate wells (Becton Dickinson, Sparks, Maryland). Each sample, blank and standard, was tested in duplicate. Fifty µL of *Limulus* amebocyte lysate was added to the samples. This mixture was mixed and incubated for 10 minutes at 37°C (98.6°F), then 100 µL of chromogenic substrate solution was

added, mixed, and incubated for an additional 6 minutes at 37°C (98.6°F). One hundred µL of 25% (v/v) glacial acetic acid in water was added to stop the reaction. Endotoxin reacted with chromogenic substrate to produce a yellow color. The absorbance of the sample was determined with a microtiter plate reader (Dynatech Laboratories, Inc., Alexandria, Virginia) at 405 nm. Serial dilutions were made on samples that exceeded 1.0 EU per mL.

For the concentration range of 0.1–1.0 EU per mL, the absorbance at 405 nm was linear. The correlation coefficient (r) for the individual mean (absorbance of the standards plotted against their respective endotoxin concentration) was ≥ 0.980.

Endotoxin levels were determined for each total, and respirable dust and control filter. The following equation was used to calculate EU per m<sup>3</sup> air:

$$\frac{\text{EU}_{\text{sample}} + \text{EU}_{\text{control}}}{1000} \times 180 \text{ minutes} \times \frac{1.7 \text{ L}}{1 \text{ minute}}$$

## Animal performance and necropsy

Animal weights (kg) were measured on an electronic scale (Mid America Scales, Inc., Tompkinsville, Kentucky) according to the following schedule:

- weight 1 taken at week 2 (weaning);
- weight 2 taken at week 4;
- weight 3 taken at week 6; and
- weight 4 taken at week 8 (necropsy).

Weight 1 was not determined in Trial 1.

At the end of 8 weeks, a necropsy was conducted on 10 pigs per group (n=40 for Trials 1 and 3; n=20 for Trial 2). Animals were euthanized with an electrical current, then exsanguinated by severing the brachial

arteries. Mesenteric, cervical, and thoracic lymph nodes; spleen; and thymus glands were collected and weighed at necropsy.

The entire thymus gland and spleen were collected.<sup>10</sup> A distinct horse-shoe-shaped area of mesenteric lymph nodes was collected from the mesentery approximately 30 cm from the ileocecal junction. The deep cervical lymph node immediately dorsal to the thymus gland was collected. The thoracic lymph node collected was located at the thoracic inlet and close to the dorsal part of the ribs.

## Statistical analysis

Probability of difference of means was calculated as part of Statistical Analysis System (SAS) general linear model procedure.<sup>11</sup> Least-squares means for describing the experimental sample were calculated using the model: treatment, group, treatment × group.

## Results

Dust concentrations (Table 1) were significantly higher ( $P < .05$ ) in the conventional nursery compared to the isolated nursery for weeks 3, 5, and 6 in Trial 1, and weeks 3, 4, and 6 in Trial 3.

Total endotoxin concentrations (Table 2) were significantly higher ( $P < .05$ ) in the conventional nursery compared to the isolated nursery for weeks 3, 5, and 7 in Trial 1; weeks 2, 4, and 5 in Trial 2; and weeks 2, 4, 5, 6, and 7 in Trial 3.

Respirable endotoxin concentrations (Table 3) were significantly higher ( $P < .05$ ) in the conventional nursery compared to the isolated nursery for weeks 5 and 7 in Trial 1; week 4 in Trial 2; and weeks 4, 5, and 7 in Trial 3.

Pigs in the isolated nursery had significantly larger thymus glands (Trial 1), spleens (Trials 1 and 2), and cervical lymph nodes (Trial 2) than animals reared in the conventional farm (Table 4). Pigs reared in the conventional nursery had significantly larger cervical lymph nodes

**Table 1**

Total dust (mg/m<sup>3</sup>) measured at two different swine-rearing facilities: isolated nursery (I), conventional farm (C)

Week	1		2		3		4		5		6		7	
	I	C	I	C	I	C	I	C	I	C	I	C	I	C
Trial 1 (n=4)	ND	ND	ND	ND	0.5*	3.4	SL	SL	1.3*	3.2	1.6*	2.6	1.8	1.7
SD	NA	NA	NA	NA	0.5	0.5	NA	NA	0.2	0.2	0.1	0.1	0.1	0.1
Trial 2 (n=2)	T	T	T	0.3	0.1	T	0.0*	2.1	T	T	ND	ND	ND	ND
SD	NA	NA	NA	0.1	0.8	NA	0.2	0.2	NA	NA	NA	NA	NA	NA
Trial 3 (n=4)	T	13.1	T	T	0.4*	11.2	0.6*	1.6	T	1.3	0.6*	2.2	T	1.6
SD	NA	2.5	NA	NA	0.5	0.5	0.1	0.1	NA	0.2	0.1	0.1	NA	0.1

ND Not determined

NA Not applicable

SL Samples lost in processing; filters destroyed during vacuum desiccation

SD Standard deviation

T Trace amounts; weight could not be determined

n number of replicates

\*  $P < .05$

**Table 2**

Endotoxin units (1 EU = 10 ng endotoxin) per cubic meter air (total dust) measured at two different swine-rearing facilities: isolated nursery (I), conventional farm (C)

Week	1		2		3		4		5		6		7	
	Treatment	I	C	I	C	I	C	I	C	I	C	I	C	
Trial 1 (n=4)	ND	ND	ND	ND	T	2.5	SL	SL	0.1*	37.1	0.1*	24.9	4.0	18.0
SD	NA	NA	NA	NA	NA	0.81	NA	NA	1.41	1.41	3.31	3.31	3.95	12.85
Trial 2 (n=2)	0.0	0.1	0.2*	4.1	T	12.5	T	43.9	T	43.9	ND	ND	ND	ND
SD	0.51	0.51	0.49	0.49	NA	0.72	NA	0.93	NA	0.93	NA	NA	NA	NA
Trial 3 (n=4)	0.0	1.8	0.1*	2.6	1.2	1.5	0.1*	13.5	0.8*	13.5	0.4*	10.3	0.5*	25.1
SD	0.02	1.60	0.31	0.31	0.81	0.81	0.12	0.12	0.12	0.12	1.41	1.41	2.34	2.34

**Table 3**

Endotoxin units (1 EU = 10 ng endotoxin) per cubic meter air (respirable fraction [ $<0.5$  microns]) measured at two different swine-rearing facilities: isolated nursery (I), conventional farm (C)

Week	1		2		3		4		5		6		7	
	Treatment	I	C	I	C	I	C	I	C	I	C	I	C	
Trial 1 (n=4)	ND	ND	ND	ND	10.8	77.1	SL	SL	63.7*	549.2	29.4	136.6	512.1*	74.7
SD	NA	NA	NA	NA	0.04	0.04	NA	NA	0.15	0.15	0.90	1.26	0.06	0.06
Trial 2 (n=2)	39.5	100.8	2.2	56.1	7.4	37.7	T	116.7	103.3	192.5	ND	ND	ND	ND
SD	0.15	0.15	0.02	0.02	0.05	0.05	NA	0.02	0.19	0.019	NA	NA	NA	NA
Trial 3 (n=4)	37.7	857.1	2.5	41.2	93.5	40.3	12.7*	343.9	30.3*	603.9	15.0	1549.0	28.7*	3456.9
SD	0.04	0.47	0.02	0.02	0.04	0.04	0.03	0.03	0.15	0.15	0.63	0.63	0.06	0.06

**Notes for Tables 2 and 3:**

ND Not determined

NA Not applicable

SL Samples lost in processing; filters destroyed during vacuum desiccation

SD Standard deviation

T Trace amounts; EU could not be determined

n number of replicates

\*  $P < .05$ **Table 4**

Comparison of lymphoid organ weights (g) obtained from pigs reared in two different swine confinement facilities: isolated nursery (I), conventional farm (C)

Organ	Thymus		Spleen		Cervical L.N.		Thoracic L.N.		Mesenteric L.N.	
	Treatment	I	C	I	C	I	C	I	C	I
Trial 1 (n=4)	163.6*	88.9	93.7*	70.1	1.8*	3.6	3.0	3.3	14.6	13.9
SD	6.2	6.5	2.4	2.6	0.2	0.2	0.2	0.2	0.8	0.9
Trial 2 (n=2)	84.0	74.6	43.6*	38.1	3.3*	1.8	ND	ND	19.5	19.8
SD	4.8	4.9	1.7	1.8	0.2	0.2	NA	NA	1.2	1.2
Trial 3 (n=4)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

ND Not determined

NA Not applicable

SD Standard deviation

\*  $P < .05$

in Trial 1. No significant differences were observed in mesenteric lymph node weights for Isoweat<sup>SM</sup> and conventional animals. (Body organ weights were not determined for pigs in Trial 3).

Body weights for Trials 1, 2, and 3 (Table 5) were significantly higher ( $P < .05$ ) for week 2, 3, and 4 in the isolated nursery animals for all weights, except week 2 of Trial 1 (conventional animals were greater in weight).

## Discussion

Although the farms in this study are not necessarily representative of all conventional or isolated facilities, the isolated nursery in this study had lower dust and endotoxin concentrations than the nurseries within the conventional farm environments. For all trials when significant differences were observed, the concentrations of dust and endotoxin were always higher in the conventional nursery, with the single exception of respirable endotoxin in the isolated nursery for week 7 of Trial 1.

In the isolated nursery, we found total dust concentrations of 0.01–2.21 mg per m<sup>3</sup> air compared to reported values of 1.1–5.1 mg per m<sup>3</sup>,<sup>12</sup> 4.9 mg per m<sup>3</sup>,<sup>13,14</sup> and 5.2 mg per m<sup>3</sup>.<sup>9</sup> We found a range of total dust concentrations of 0.12–13.09 mg per m<sup>3</sup> in the conventional nursery.

Total endotoxin level findings ranged from 1217–439,322 ng per m<sup>3</sup> air in the conventional nursery and from 197–4135 ng per m<sup>3</sup> in the isolated nursery. Reported values of total endotoxin, 120 ng per m<sup>3</sup>, 310 ng per m<sup>3</sup>,<sup>15</sup> 24–59 ng per m<sup>3</sup>,<sup>16</sup> 3040 ng per m<sup>3</sup>,<sup>17</sup> and 62.7 ng per m<sup>3</sup>,<sup>13</sup> are lower than those found in these three trials. Levels of total endotoxin in the isolated nursery compare to the above reported values in all but one of 16 data points; otherwise, they are within published ranges. Concentrations of total endotoxin in the conventional nursery are higher than the reported values except on 5 of 16 data points, which are within published ranges. Respirable endotoxin levels in both the isolated nursery (22.4–5,121 ng per m<sup>3</sup>) and conventional nursery (377–34,569 ng per m<sup>3</sup>) are consistently higher compared to published data, 3.8–9.8 ng per m<sup>3</sup>.<sup>16</sup>

Differences noted in total and respirable endotoxin concentrations may be due to different techniques for measuring concentrations of endotoxin. Attwood, et al., and Baekbo used the same endotoxin assay; however, they used different methods to collect endotoxin.<sup>13,16</sup> Attwood, et al., determined concentrations of endotoxin from glass

microfiber filters, whereas Baekbo conducted endotoxin analysis on a collection media.

Acceptable concentrations for humans and animals have been recommended for dust (humans: 2.4 mg per m<sup>3</sup> air, pigs: 3.7 mg per m<sup>3</sup> air) and various gases.<sup>18</sup> In the conventional nursery, concentrations of respirable dust were higher than recommended values for humans in 5 of 16 samplings, and exceeded recommended concentrations for pigs in 2 of 16 samplings.

The improved pig performance observed in Isoweat<sup>SM</sup> pigs for all three trials is consistent with previously published data.<sup>7</sup> The significantly heavier thymus glands of pigs in the isolated nursery compared to animals in the conventional nursery for Trial 1 is also consistent with previously published data.<sup>8</sup>

Significant differences in dust and endotoxin were observed between the isolated nursery and conventional farm. Pig growth performance and lymphoid organ weights were also different; therefore, an association between airborne contaminants and pig performance may exist. Several reasons may have contributed to the differences in concentrations of dust and endotoxin. The second-stage conventional nursery shared a common pit with other second-stage nursery rooms within the farm; therefore, air exchange between nursery rooms may have occurred. Manure pits in the isolated nursery rooms were completely separate, preventing any air exchange between rooms.

Another difference was in management of the facilities. The manure pit in the isolated nursery was flushed two to three times per week, whereas the manure pit in the conventional nursery was drained only when pigs were removed from the nursery. The conventional nursery was pressure washed when the pigs were removed, before the room was refilled; however, residual manure remained in the manure pit. The rooms in the isolated nursery were also pressure washed when the

**Table 5**

Mean weights (kg) by week placed of pigs reared in two different swine confinement facilities: isolated nursery (I), conventional farm (C)

Week	2		4		6		8	
	I	C	I	C	I	C	I	C
Trial 1, group 1	5.1	ND	6.7*	6.2	12.8*	11.4	32.4*	21.5
SD	0.0009	NA	0.0013	0.0011	0.0024	0.0028	0.0042	0.0045
Trial 1, group 2	5.5	ND	5.1*	7.9	11.6*	9.9	26.6*	17.7
SD	0.0006	NA	0.0009	0.0012	0.0019	0.0016	0.0029	0.0024
Trial 2 (n=2)	6.2	6.2	11.4*	10.3	19.9*	16.8	23.6*	21.5
SD	0.0011	0.0010	0.0016	0.0018	0.0026	0.0032	0.0031	0.0037
Trial 3, group 1	4.9*	5.0	7.6*	6.3	15.1*	10.7	24.5*	17.4
SD	0.0008	0.0008	0.0010	0.0009	0.0021	0.0015	0.0034	0.0030
Trial 3, group 2	4.7*	5.0	8.4*	6.6	15.5*	11.8	25.3*	19.5
SD	0.0006	0.0008	0.0014	0.0012	0.0020	0.0024	0.0027	0.0031

ND Not determined  
 NA Not applicable  
 SD Standard deviation  
 \*  $P < .05$

pigs were removed, before the room was refilled; however, no residual manure remained in the manure pits. Restricted feed for 2 weeks at the conventional nursery versus ad libitum feeding in the isolated nursery may have also affected weight gain. This is the first report of environmental differences being associated with biological performance differences.

Although the results of these experiments suggest a role for endotoxin in depressing the growth rate of pigs, other confounding factors not considered in this experimental design may have affected growth performance. For example, the total room air volume was markedly different between the conventional farm nurseries and the isolated nursery. In addition, the isolated nursery was a one-stage system while the conventional nursery was two-stage system. There was also a two week feed restriction in the conventional nursery. Finally, swine pathogen loads may have differed between the groups. Therefore, these results should be considered to be preliminary and require further study.

## Implications

- In this study, we found an association between low facility dust and endotoxin concentrations and improved growth rates in pigs reared by Isowean<sup>SM</sup>.
- Offsite early weaning in this study was associated with improved performance and large thymus size.
- Confounding factors existed in the study; therefore, causal relationship cannot be identified at this point. Further research to clarify confounding factors is needed.

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