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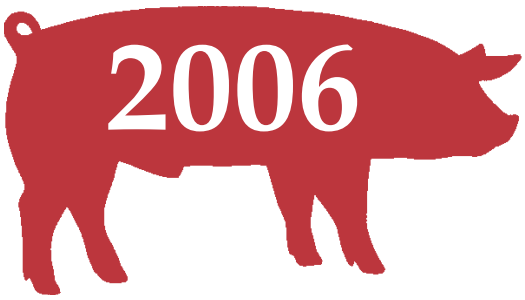

NEBRASKA SWINE REPORT

- Nutrition
- Genetics
- Management
- Reproduction
- Odor

Web site:
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Prepared by the staff in Animal Science and cooperating Departments for use in Extension, Teaching and Research programs.

Cooperative Extension Division
Agricultural Research Division
Institute of Agriculture and Natural Resources
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Cover Photo:

Marcelo Montagner, Brett White and Ginger Mills hold the piglets produced from frozen embryos. ***Photo by Duane Reese***

The 2006 Nebraska Swine Report was compiled by Duane Reese, Extension Swine Specialist, Department of Animal Science.

2006 Nebraska Swine Report

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Impact of Repeated Out-Of-Feed Events and Fineness of Grind on Grow-Finish Performance

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Summary and Implications

Out-of-feed events are a growing problem in nursery and grow-finish facilities due to issues associated with feed delivery to bulk bins and bridging of feed in bulk bins. Reports of bridging are increasing as producers continue to reduce the fineness of grind for complete diets in order to improve feed conversion. A study was conducted to examine the effect of repeated out-of-feed events and diet particle size on barrow performance in a wean-to-finish facility beginning six weeks after weaning. Corn-soybean meal based diets were either coarse (1,266 microns) or medium ground (1,019 microns) for the duration of the experiment. Within particle size, pigs were never out-of-feed or denied access to feed for a 20-hour period beginning at noon on a random day each week for 16 weeks. For the first eight weeks, weekly out-of-feed events reduced daily gain 0.15 lb/day compared to the never out-of-feed treatment (P<0.001) due to a reduction in daily feed intake (P=0.003) with no effect on feed conversion efficiency. There was no effect of out-of-feed events on daily gain or feed conversion for the second eight week period of the experiment. For the 109-day trial period, weekly random 20-hour out-of-feed events resulted in a 0.077 lb/day decrease in daily gain with no effect on feed conversion. The 247 micron reduction in average diet particle size resulted in a 0.091 lb/lb improvement in overall feed conversion (P=0.001) for the coarse versus medium ground diets. There was no effect of any experimental treatment on skin lesion scores, a measure of pig welfare and injury from fighting at the feeder. There was no interaction of out-of-feed events and diet particle size. These results suggest that out-of-feed events can have major consequences for pig performance. However, pigs appear to adjust to weekly out-of-feed events, even when they occurred on a random day within each week. The penalty for repeated out-of-feed events is a reduction in daily gain, with no impact on feed conversion, while the penalty for coarser ground diets is a worsening in feed conversion, with no change in daily gain.

Introduction

In theory, bulk bins and automated feed delivery systems assure an uninterrupted flow of feed to feeders in swine grow-finish facilities. In practice, growing-finishing pigs have varying disruptions in feed availability, some of which may have very serious consequences. While every swine grow-finish facility has occasional disruptions due to mechanical failures in the feed delivery system, there are additional disruptions due to human errors associated with delivering feed to the bulk bin and feed bridging associated with feed removal from the bin. Out-of-feed events are a known cause of ulcers in pigs and are suspected of being associated with increased incidence of hemorrhagic bowel syndrome and ileitis. It has been speculated that each 20 to 24 hour out-of-feed event results in an increase in variation in growth within a population of pigs and results in a reduction in daily gain.

Pork producers routinely mill ingredients in swine diets to have a particle size of 700 to 900 microns, because the finer particle size results in better feed conversion efficiency. Recent results from Kansas State University suggest that as particle size decreases, and the amount of fat added increases in corn-based diets, the angle of repose (an estimate of likelihood of bridging) increases. Data suggest a 1-1.5% improvement in feed conversion efficiency for each 100 micron reduction in particle size from 1000 to 500 microns. The current University of Nebraska recommendation is to process complete diets to an average particle size of 650 to 750 microns for all grains except wheat.

The following experiment was designed to examine the interaction of fineness of grind and random out-of-feed events on pig performance and welfare.

Materials and Methods

The research was conducted at the University of Nebraska's Haskell Ag Lab at Concord. The research facility was a fully slatted, naturally ventilated wean-to-finish unit with 16 pens (8 ft x 14 ft). Each pen had one, two-hole Farmweld wean-to-finish feeder and one Drik-o-Mat wean-to-finish cup drinker. There were 15 pigs per pen at weaning (7.5 ft^2/pig) and

(Continued on next page)

pen size was not reduced in the event of pig death or removal.

On the day of weaning (14-21 days of age), the pigs were transported approximately 200 miles. At arrival, they were ear tagged, individually weighed and assigned to pens on the basis of arrival weight such that all pens had similar mean weights and within pen coefficients of variation for weight. The experimental treatments began six weeks after weaning. There were four pens of pigs per experimental treatment combination. Only barrows were used in this experiment to minimize the random out-of-feed events associated with gilts urinating in a feed trough and plugging a feeder for an unknown length of time.

The experimental treatments, in a 2 x 2 factorial arrangement, were:

1. Out-of-feed, never or weekly,
2. Feed particle size, coarse or medium.

The out-of-feed events consisted of closing the feeder delivery device completely at noon and reopening the device at 8:00 the following morning which resulted in a 20-hour period when no feed was available to a pen of pigs. The day of the week that the out-of-feed event began was randomly selected each week from Monday through Friday. Pigs were weighed every other week, and on the week of weighing, the feeders were never closed on Thursday evening so that pig weights on Friday morning were not confounded with an out-of-feed event.

Corn-soybean meal based diets were formulated with corn ground in a full-screen hammer mill with two different screen sizes. Feed samples were collected every other Friday, stored, and submitted for particle size analysis at the conclusion of the experiment.

Diets containing 40 g/ton tylosin were switched to the next lysine sequence on the basis of the average weight of all pigs in the facility. Lysine levels were 1.15% from 45-80 lb, 0.99% from 80-135, 0.77% from 130 to 195, and 0.62% from 195 to slaughter. Diets contained 3% added fat from 45 to 135 lb body weight and 1.5% added fat thereafter.

Skin lesions (i.e. lesions that were pink/bleeding), tail biting, and lameness were observed on every weigh day and independently scored by two observers. Lesions were ranked on a 0 to 4 scale with 0 being no fresh lesions observed and 4 being many (12+ small or 6+ large) lesions. Tail biting was ranked on a 0 to 4 scale with 0 being no tail biting and 4 being a large, deep and open wound.

Pigs were vaccinated for erysipelas, M. hyo and ileitis prior to the start of the out-of-feed events. All pigs that died were examined by a veterinarian for cause of death. Prior to the out-of-feed events, pigs were diagnosed with Strep suis and gut edema, most likely caused by a beta-hemolytic *E. coli*.

All pigs that weighed greater than 205 lb were slaughtered at Tyson Foods in Madison, Neb., four days after final weights were taken. Pigs were tattooed by pen, and pen average carcass data for back fat, loin muscle depth and percent lean was provided by Tyson Foods.

The pen of pigs was the experimental unit for all observations.

Results and Discussion

The only interactions ($P<0.1$) between feed particle size and out-of-feed events was starting weight and carcass lean percent. Thus, the main effects of the experimental treatments on pig performance are presented in Table 1.

Random, weekly 20-hour out-of-feed events resulted in a 0.077 lb/day reduction in daily gain ($P=0.008$) compared to pigs that were never out-of-feed. Weekly out-of-feed events reduced daily feed overall 0.195 lb/day ($P<0.023$) but had no effect on feed conversion.

The pigs adapted to the random weekly out-of-feed event. For the first 53 days of the experiment, daily gain was reduced 0.150 lb/day compared to 0.009 lb/day for the subsequent 56-day period for the out-of-feed versus never pigs. Similarly, daily feed was reduced 0.291 lb/day for the first 53-day period and only 0.101 lb/day for the subsequent period. There was no difference in feed conversion between the out-of-feed and never treatments for either period. Figures 1 and 2 document the declining impact of the out-of-feed events on daily gain and daily feed intake as the trial progressed.

Because of the overall reduction in daily gain, out-of-feed pigs were lighter at slaughter, had lower hot carcass weights, carcass fat depth and carcass loin depth compared to the never pigs. There was no effect of out-of-feed events on carcass lean percent.

Particle size for the medium treatment was coarser than expected, even though ground corn was pre-sampled at the commercial mill for both particle sizes with the intent of having coarse and fine particle size diets. Particle size for the coarse diet averaged 1,266 microns (2.16 SD) and that for the medium diet averaged 1,019 microns (1.61 SD) for the entire trial. However, for the first eight weeks, particle size for the coarse and medium diets was 1,224 microns (2.4 SD) and 929 microns (1.7 SD). For the second eight-week period, the corresponding particle sizes were 1,307 microns (1.9 SD) for the medium diet and 1,109 microns (1.6 SD) for the coarse diet.

The response to differences in diet particle size agrees with previously published results. There was no effect of particle size on daily gain. However, pigs fed the coarse diets ate more feed for the final 56-

Table 1. Impact of experimental treatments on pig performance.

	Out of feed[a]		Particle size[b]			P values		
Item	Never	Weekly	Coarse	Medium	SE	OOF	PS	OOF x PS
No. pens	8	8	8	8				
Pig wt, lb								
On test	53.2	51.3	52.3	52.2	0.7	0.074	0.941	0.031
Day 53	155.2	145.3	150.9	149.6	1.7	0.001	0.613	0.197
Day 109	261.8	251.5	257.6	255.7	2.3	0.007	0.559	0.274
Coefficient of variation of pig weight within pen, %								
On test	17.4	16.1	16.3	17.3	1.6	0.891	0.099	0.301
Day 53	10.7	10.9	10.3	11.3	1.0	0.881	0.513	0.997
Day 109	8.1	8.2	7.5	8.8	0.7	0.881	0.230	0.475
Daily gain, lb								
On test - day 53	1.924	1.774	1.860	1.838	0.022	<0.001	0.482	0.638
Day 53 - day 109	1.903	1.894	1.905	1.891	0.019	0.746	0.612	0.246
Overall	1.913	1.836	1.883	1.866	0.017	0.008	0.515	0.651
Daily feed, lb								
On test - day 53	4.421	4.130	4.344	4.208	0.056	0.003	0.113	0.552
Day 53 - day 109	6.566	6.465	6.674	6.358	0.058	0.240	0.002	0.525
Overall	5.524	5.329	5.541	5.311	0.053	0.023	0.010	0.497
Feed:gain								
On test - day 53	2.298	2.328	2.335	2.290	0.013	0.133	0.032	0.601
Day 53 - day 109	3.448	3.413	3.501	3.359	0.022	0.291	0.001	0.700
Overall	2.888	2.901	2.940	2.849	0.016	0.545	0.001	0.612
Carcass data, Tyson Fresh Meats, Madison, Neb.								
Carcass wt, lb	206.3	197.5	201.6	202.1	1.7	0.004	0.839	0.173
Fat depth, in	1.02	0.97	0.98	1.00	0.02	0.146	0.518	0.114
Loin depth, in	2.75	2.67	2.72	2.70	0.02	0.023	0.553	0.306
Lean, %	53.5	53.5	53.6	53.4	0.1	1.000	0.257	0.021
Pigs dead, no.[c]	2	4	2	4				
removed, no.	1	1	0	2				
< 205 lb, no.	2	4	2	4				

[a]Never = never out-of-feed; Weekly = 20 hour out-of feed on a random day each week.
[b]Coarse = average 1,266 microns; Medium = average 1,019 microns.
[c]Chi Square analysis; P > 0.1 for all comparisons.

day period compared to pigs fed the medium ground diets. Pigs fed the coarse diets had poorer feed conversion efficiencies for both the 53-day initial and 56-day final period. This resulted in a 0.091 lb of feed per lb of gain improvement in overall feed conversion efficiency for the pigs fed the medium versus coarse diet (P<0.001), an improvement of 3.1%.

Feed was delivered in bulk for this experiment and augered into a weigh cart for delivery to individual feeders. While not quantified, there were considerably fewer bridging problems with feed removal from the bulk storage bins for the coarse versus medium diets.

Because of the small numbers involved, it was not possible to detect a significant difference between treatments for the number of pigs that died, were removed or weighed less than or equal to 205 lbs at the end of the experiment. However, pigs fed the medium diets and pigs experiencing weekly random out-of-feed events had numerically higher numbers of deaths and lightweight pigs at the end of the experiment. Two pigs were removed from the experiment for severe tail biting injury with one on the medium/out-of-feed treatment combination and the other on the medium/never out-of-feed treatment combination.

There was no effect (P > 0.1) of any experimental treatment on skin lesions scores (Table 2). There was also no effect (P > 0.1) of out-of-feed events on tail biting. However, pigs fed the medium diet had an increase in severity of tail biting score compared to the coarse pigs (P=0.012).

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Conclusion

Weekly, random 20-hour out-of-feed events reduced overall gain at the end of a 109-day grow-finish trial 11.3 pounds. With 16 out-of-feed events, this amounted to a 0.7 lb decrease in gain for each out-of-feed event overall. However, the main impact of out-of-feed events occurred in the first eight weeks, when daily gain was reduced 0.15 lb/day for a total gain depression of 7.9 lb for pigs experiencing weekly, random out-of-feed events. There was no effect of out-of-feed events on feed conversion efficiency, nor was there any interaction between out-of-feed events and feed particle size. As expected, pigs fed the medium ground diets had no difference in daily gain, but had a 3.1% improvement in feed conversion efficiency compared to pigs fed the coarsely ground diets. In production units that must sell pigs by a certain date, these data will allow producers to examine whether the improvement in feed conversion efficiency from finely ground diets overcomes the loss in weight gain from out-of-feed events that may be due to increased bridging of finely ground diets.

[1]Michael C. Brumm is a professor of animal science and extension swine specialist, and Sheryl L. Colgan is a research technologist at the Northeast Research and Extension Center in Concord, Neb. This research was financially supported by a grant from the Nebraska Pork Producers Association through the National Pork Board.

Table 2. Impact of experimental treatments on skin lesions and tail biting score (0 to 4 scale).

	Out of feed[a]		Particle size[b]		P value[c]	
Item	Never	Weekly	Coarse	Medium	OOF	PS
Average skin lesions score	0.29	0.26	0.27	0.27	0.351	0.808
Average tail biting score	0.03	0.02	0.01	0.05	0.301	0.012

[a]Never = never out-of-feed; Weekly = 20 hour out-of feed on a random day each week.
[b]Coarse = average 1,266 microns; Medium = average 1,019 microns.
[c]Friedman Chi Squared test on ranked pen means.

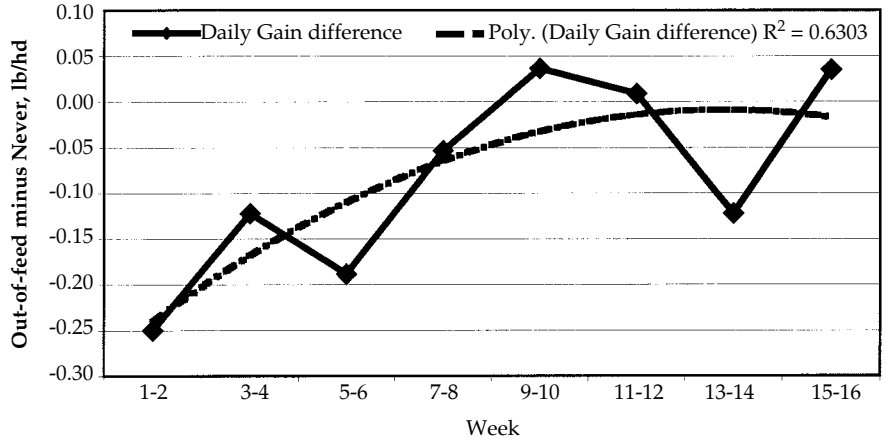

Figure 1. Reduction in daily gain by two-week period for the out-of-feed treatment versus the never out-of-feed treatment.

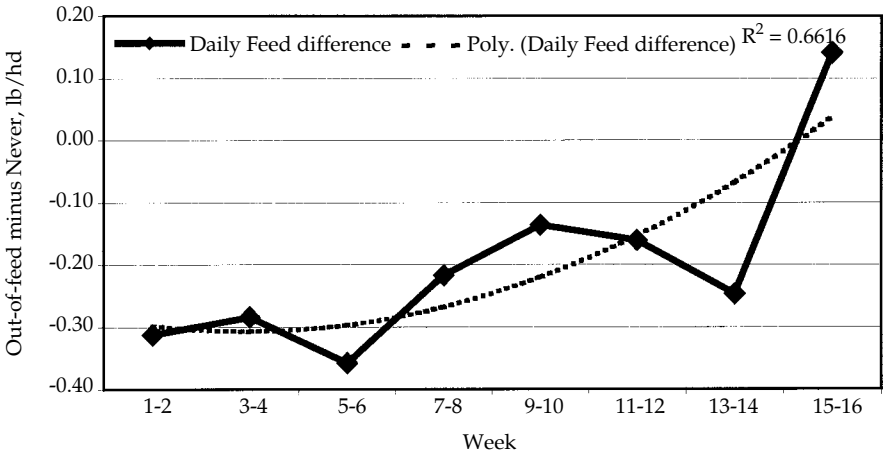

Figure 2. Reduction in daily feed by two-week period for the out-of-feed treatment versus the never out-of-feed treatment.

The Case Against Evening-Up Litters Until Weaning

Duane E. Reese
Barbara E. Straw[1]

Summary and Implications

A literature review on the effect of fostering or moving individual piglets from one litter to another after they are 24 to 48 hours of age was conducted. Late fostering disrupts nursing, increases fighting, and impairs the growth rate of adopted piglets and their littermates. Pig body weight at weaning was reduced 13 to 24% in extensively fostered litters vs. those where no piglets were fostered after 48 hours of age. No evidence was found that late fostering improves preweaning survival. For the greater good of all piglets, producers are encouraged to resist the urge to even-up litters or foster individual piglets after they are 24 hours old. Piglets that fall behind or grow slower than littermates after the initial fostering is done should be transferred to nurse sows where an entirely new litter(s) of older pigs is made. Milk replacers can also play a role in providing slower-growing or starving piglets more milk.

Introduction

Fostering or moving piglets from one litter to another is commonly practiced in swine operations to adjust litter size so that all piglets have good access to sow milk during lactation. Proper fostering reduces preweaning mortality and probably the number of substandard pigs at weaning. Many farrowing managers and employees know all fostering should be completed before the piglets are 24 to 48 hours old for best results. However, in some operations, moving individual piglets between litters or "evening-up" continues until weaning. Some farrowing house personnel hate to see one litter with 10 pigs and the one next to it with seven. Also, some believe that a piglet in one litter that is falling behind littermates would be better off living in another litter of more similar-sized piglets, especially if there are fewer piglets in the recipient litter. Basically the goal is to have all litters in the farrowing area uniform or looking like "peas in a pod." This paper will review the literature on fostering to clarify the issue for people who continue to even-up litters until weaning. Also, options for accommodating fall-outs or piglets that grow slower than littermates before weaning will be presented.

Research Summary

Michigan State University researchers conducted a study with 80 litters on a farm where extensive transfer of pigs between litters was being done on a daily basis until weaning. In 40 litters the usual practice of continuous fostering until weaning was continued. In another 40 litters fostering was limited to the first two days of life.

The effect of extensive fostering on pig body weight and standard deviation of body weight at weaning and preweaning mortality is presented in Table1. As expected, extensive fostering resulted in a lower average within-litter standard deviation of pig body weight at weaning (i.e., pigs were more uniform in size within litter); however, it also reduced pig weaning weight by 2.2 lb or 20%. Mortality was not significantly different between treatment groups, although it was numerically higher in the continuous fostering treatment. This research demonstrated that continuous fostering results in more uniform litters at weaning, but at the expense of growth rate and possibility survival.

In half of the 32 litters Canadian researchers studied, two piglets were exchanged between pairs of litters at 6 $\pm$ 1 day of age. Thus, there were three types of piglets in the study; adopted (piglets that were exchanged), resident (piglets that were not exchanged but were littermates to the adopted piglets), and control (no fostering). Piglets were weighed at birth, fostering, weaning (day 18 $\pm$ 1) and weekly during the next month.

The effect of fostering on pig body weight during the course of the trial is shown in Figure 1. There was no significant difference in body weight between piglet types at birth or just before fostering. However, at every time period after fostering, the average body weight of piglets in the fostered litters (those containing adopted and resident piglets) was significantly

(Continued on next page)

Table 1. Effect of limited vs. continuous fostering on pig performance during lactation.[a]

Item	Limited fostering[b]	Continuous fostering
Average within-litter standard deviation of body weight at weaning[c]	2.0	0.7
Weaning weight, lb[c]	11.6	9.4
Mortality, %	8.0	8.8

[a]Straw et al., 1998.
[b]First two days of life only.
[c]P<0.008.

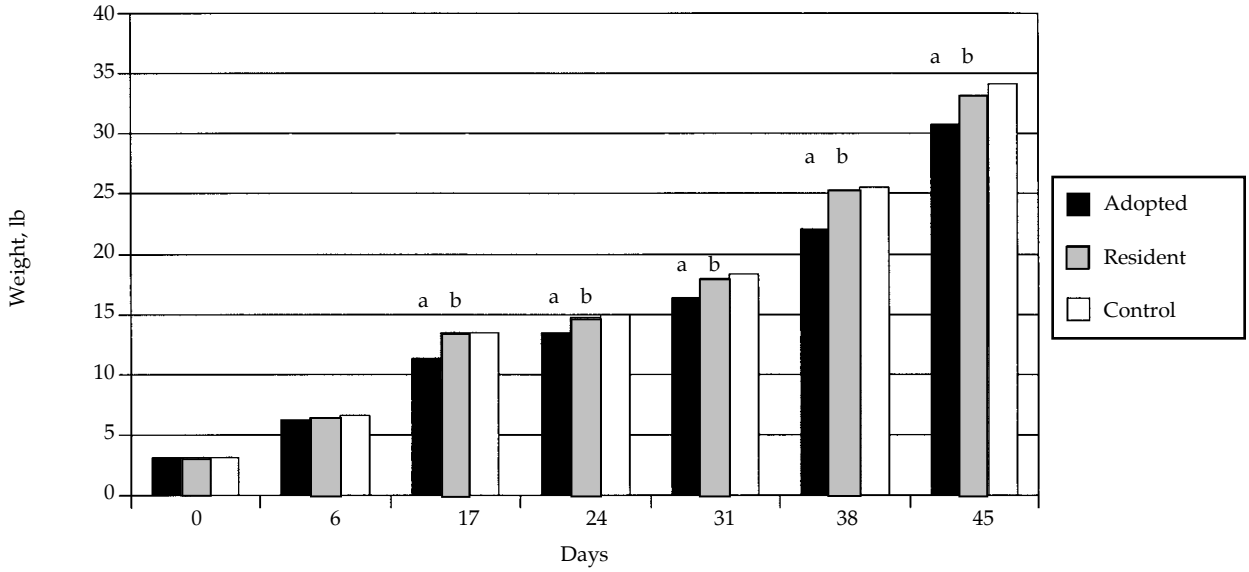

Figure 1. Effect of fostering on day 6 ± 1 of lactation on piglet body weight before and after weaning (day 18 ± 1). [a]Body weight between fostered (adopted + resident) and control litters differed (P < 0.05); [b]Body weight between adopted and resident piglets differed (P < 0.05). Adapted from Giroux et al., 2000.

reduced. Within the fostered litters the body weight of adopted piglets was significantly reduced compared to that of the resident piglets at each period. In conclusion, fostering had a marked effect on the growth rate of adopted piglets such that they gained only 76% of the weight of those in stable litters. While supporting the results of the Michigan State study, this research further demonstrated that adopted piglets may continue to be smaller after weaning.

In another Canadian study the behavior and growth of 13 control and 14 fostered litters was compared. Once every three days (from day one to 16 of lactation), all piglets were weighed and three piglets were switched between two fostered litters. Thus, there were three types of piglets in the study: adopted (piglets that were exchanged), resident (piglets that were not exchanged but were littermates to the adopted piglets), and control (no fostering). Behavior was observed for two hours after weighing and (or) fostering and during one nursing period 24 hours later.

Fights were significantly more frequent in the fostered vs. control litters during and between nursings at all fostering periods except on day one. Most of the fights occurred between resident and fostered piglets except on day 1. While nursing, piglets in fostered litters fought significantly more than those in control litters at 24 hours after fostering except on day one and 16. Failed nursings and snaps by the sow toward piglets were significantly more frequent in fostered vs. control litters. Moreover, sows rearing fostered litters spent 15 to 30% less time lying on their sides at day four, seven, 13 and 16 ($P < 0.05$). Adopted piglets weighed 13% less than controls at weaning ($P < 0.01$); resident piglets were significantly heavier than adopted piglets, but smaller ($P = 0.1$) than controls.

This study provided insight into why continuous or late fostering reduces piglet weight gain. The presence of alien piglets in the litter disrupts nursing and therefore milk intake, not only as a result of fighting between piglets, but the sow is less accommodating to the nutritional and comfort needs of her litter. The study also confirmed that fostering is appropriate through the first day of life.

Better Management Options

It's common for one or more piglets in a few litters to fall behind or grow slower than littermates during lactation. These piglets are commonly called fall-outs, fall-backs, or runts. Many fall-outs will flourish once they have the opportunity to receive more milk. Producers can use other sows (i.e., nurse sows) and(or) milk replacers to provide fall-outs more milk.

Nurse sows

Nurse sows can be created and utilized two ways. The preferred method is to identify a well-milking sow(s) to raise fall-outs that are collected from other sows. The procedure involves finding a newly farrowed gilt, i.e., one that finished farrowing six to 12 hours earlier. It is better to use a gilt, because she has smaller teats that are easier for piglets to grasp. Being newly farrowed is an advantage, because she doesn't know that the pigs she is about to receive

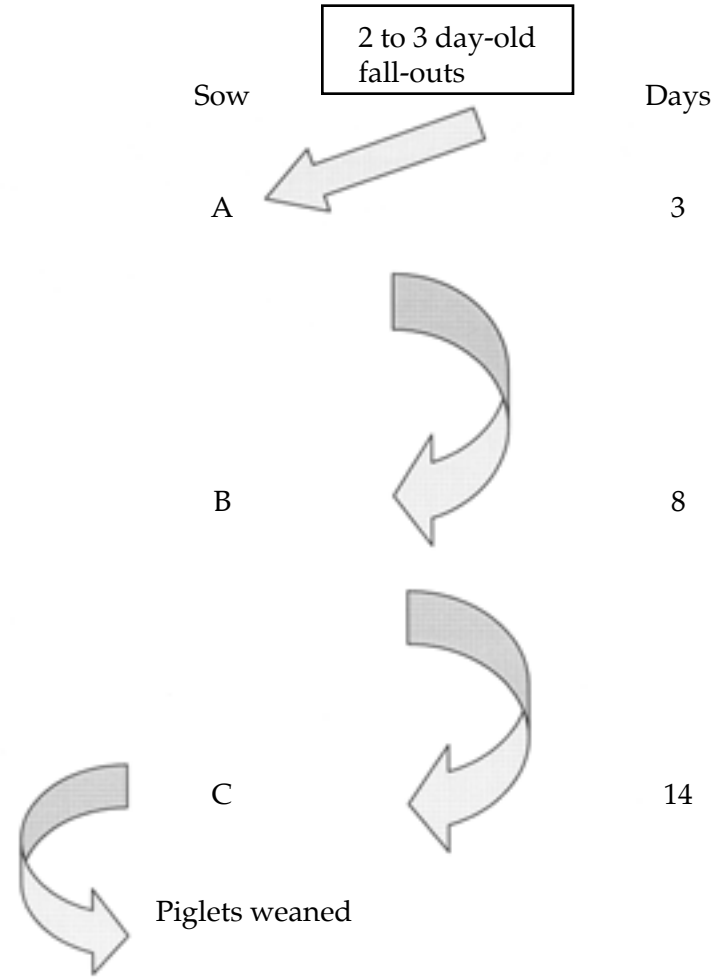

Figure 2. Example of bump weaning.

aren't her pigs. Identify eight to 10 fall-out pigs that are five to seven days of age and move them to the freed-up gilt. This moves them to a younger age group, but they are likely just starving and not sick. To be sure fall-outs are just starving and not sick, check their littermates to see if they are healthy. Also, observe if the fall-outs are being crowded out at the udder or are nursing a lower-producing teat (usually located at the rear of the udder). You do not want to move sick piglets, because that spreads disease around. The difficulty with this method is having enough spare sow capacity to take care of the gilt's original litter. It is important to wait six to 12 hours after the gilt has finished farrowing before her piglets are fostered to other sows to ensure all her piglets receive a good dose of colostrum. Remember to foster the gilt's piglets to other sows that are nursing similar-aged piglets.

The other procedure, commonly called "bump weaning," involves moving fall-outs to a later lactation, good milking sow until they reach the normal weaning age for the farm (Figure 2). For example, assume there are three good-milking sows: sow A has lactated for three days, sow B has lactated for eight days and sow C has lactated for 14 days (five to seven days before she will be weaned). Sow C's piglets are weaned and Sow B's piglets are moved to Sow C. Sow A's piglets are moved to Sow B. Sow A is given 2 to 3 day-old fall-outs collected from several litters. The main disadvantage with this procedure is that too often one or two adopted piglets per litter get injured by vicious sows. Ultimately, however, the detriment to the bumped pigs is probably outweighed by the benefit to the fall-outs (which were likely to die without some milk). For these reasons, bump weaning should be used as a last resort instead of a routine procedure. Bump weaning ensures that no piglets will be weaned later than the age limit set for the farm and that entire rooms of sows and litters can be weaned at the same time. Note that pigs are always moved forward and not backward in the system. Also, the key to making bump weaning work is to identify candidate pigs early in lactation (2 to 3 days of age) rather than later.

Milk replacers

Milk replacers offer another way for fall-outs to obtain more milk. Milk can be provided free choice in plastic milk feeders or baby bottles. Or you can place the fall-out in a plastic bin containing a feeder while it drinks. This method ensures the fall-out is not competing for milk and you can be sure it drinks. Initially the fall-out must be trained to drink from a bottle, but after a few feedings it catches on and takes advantage of the additional milk without competition. Fall-outs can also consume milk from a pan or bowl; some will need to be trained, however. To train fall-outs to drink from a bowl, place their snout in the milk for a few seconds every hour until they appear to have learned to drink on their own. Use bowls or pans that attach to or are held down to the floor so they cannot be knocked over.

Some producers place a deck or pen containing a milk feeder in each farrowing room (one deck per 12 crates, for example) to manage fall-outs. The best milking sow that has lactated for about 10 days is

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identified. Her pigs are removed and placed in the deck and fed milk replacer. Eight to 10 fall-outs are collected from various litters in the room and placed on the newly weaned sow.

Conclusion

Fostering piglets after they are 24 hours old disrupts nursing, increases fighting, and significantly impairs the growth rate of adopted piglets and their littermates. Also, no evidence was found that late fostering improves preweaning survival. Therefore, for the greater good of all piglets, resist the urge to even-up litters or foster individual piglets after they are 24 hours old. Piglets that fall behind or grow slower than littermates after the initial fostering is done should be transferred to nurse sows where an entirely new litter(s) of older pigs is made. Milk replacers can also play a role in providing fall-outs more milk.

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Patterns of Drinking Water Use in Pork Production Facilities

Michael C. Brumm[1]

Summary and Implications

The amount of drinking water needed daily by the pig depends on numerous influences, including temperature, diet, stage of production and health. Within a 24-hour period under thermal-neutral conditions, grow-finish and gestating swine demonstrate a peak in water usage in late afternoon while lactating females consume water more consistently throughout the day. In times of heat stress, grow-finish pigs alter their water usage pattern with a peak between 8 to 9 a.m. and second peak around 5 to 8 p.m. Daily drinking water needs for pigs range from less than 0.5 gal/pig/day for newly weaned pigs to greater than 1.5 gal/pig/day for grow-finish pigs using nipple drinkers. Water requirements for breeding swine range from 3 to 4 gal/day for gestating females and 6 gal/day for lactating swine. Knowledge of the daily water needs of pigs, and the patterns of water usage within the day allow for the appropriate sizing of delivery devices and prediction of the impact of pork production on available water supplies. Daily charting of drinking water usage can serve as a predictor of the on-set of swine health challenges such as swine influenza. As more sophisticated methods become available to record water usage, other predictors of performance may be developed depending on the patterns detected.

Introduction

With the on-going drought in central and western Nebraska and the controversy surrounding the environmental impact of pork production facilities, a basic understanding of the water usage patterns in pork production facilities is important. In addition, deviations from normal patterns may be a predictor of health and future performance.

How much water does a pig drink?

Daily drinking water needs for pigs range from less than 0.5 gal/pig/day for newly weaned pigs to greater than 1.5 gal/pig/day for grow-finish pigs using nipple drinkers in warm conditions. Grow-finish pigs using bowl/cup drinkers or wet/dry feeders use less water, generally averaging just over 1.0 gal/pig/day. Water requirements for the breeding herd range from 3 to 4 gal/day for the gestating female to 5 to 6 gal/day for the lactating female.

Using the above numbers, it is possible to predict the yearly water usage by various pork production facilities. For example, a 1,000 head grow-finish facility typically has a pen space utilization rate of 85-90%. That is, there are pigs occupying pen spaces 310 to 330 days per year. If the facility has nipple drinkers and a 90% facility utilization rate, total drinking water use for the facility will be:

1,000 spaces x 330 days/year x 1.5 gal/space/day = 495,000 gal

While 495,000 gallons of water seems like a big number, when compared to the water used for irrigated crop production, it is minor. An acre-inch of water (an inch of water covering an acre of ground) is equivalent to 27,154 gallons of water. This means the example finisher will use just over 18 acre-inches of water.

If drinkers that have been proven to waste less water are used such as bowl drinkers or wet/dry feeders, total drinking water use for the facility is estimated to be:

1,000 spaces x 330 days/year x 1.05 gal/space/day = 346,500 gal

This equates to 12.8 acre-inches of water.

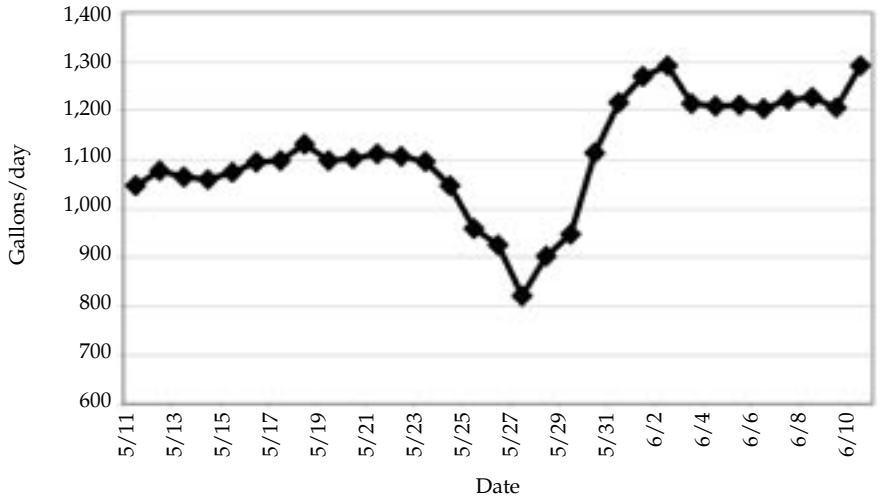

Figure 1. Impact of swine flu on daily water usage in a 860-head fully slatted finishing facility in Nebraska. Data courtesy Dicamusa.com.

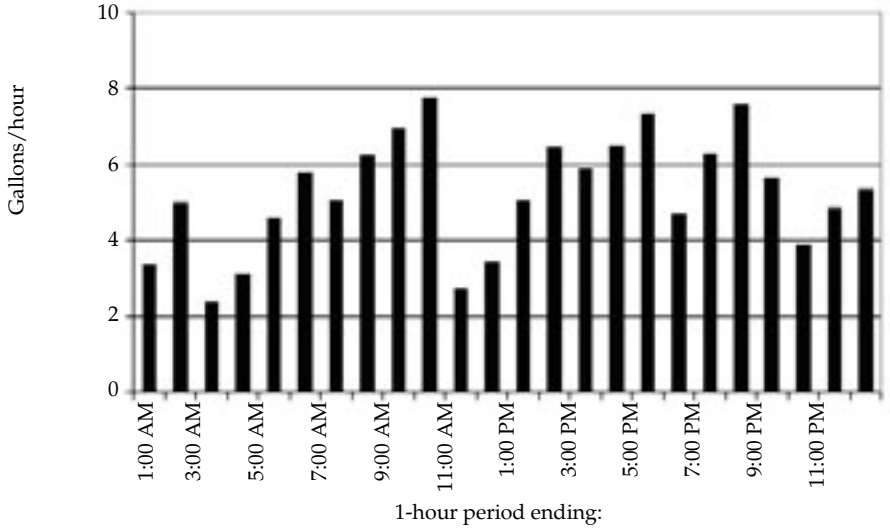

Figure 2. Hourly drinking water use in a 24-crate farrowing room for one 18-day lactation period beginning March 5. Data courtesy Dicamusa.com.

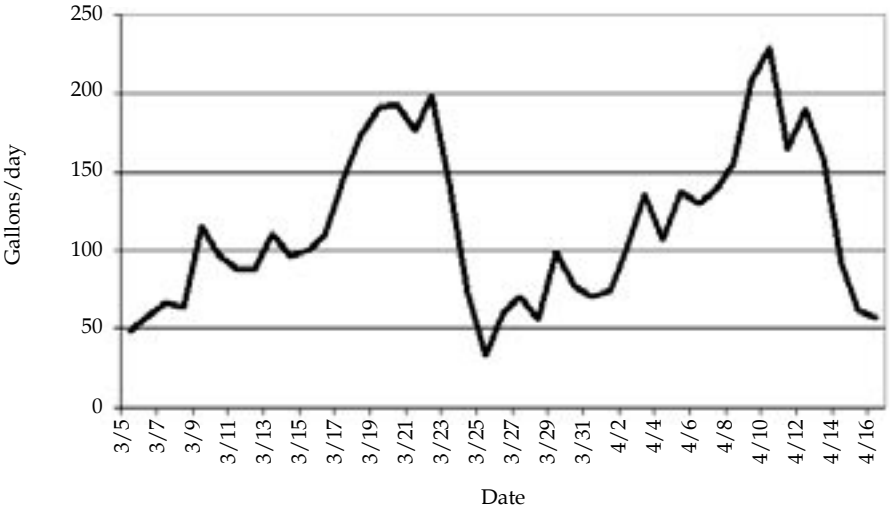

Figure 3. Daily drinking water use in a 24-crate farrowing room over two lactations. Data courtesy Dicamusa.com.

To put this amount of water in perspective for rural communities, consider that corn production typically requires 22 to 25 acre-inches of water per year. Of this, some is supplied by rain with the remainder supplied by irrigation for much of Nebraska's production. In central and western Nebraska, irrigators often supply 12 to 15 acre-inches of water to maximize yield and prevent drought stress. This suggests that a 1,000 head swine finishing facility using drinkers that are known to waste water uses less water than the amount of water used to irrigate 2 acres of corn per year.

Is there a relationship between daily water consumption and pig health?

Producers are becoming aware of the relationship of drinking water usage and pig health. Figure 1 depicts the impact of swine flu on daily water disappearance in a fully slatted 860-head finishing facility in Nebraska six weeks after pig placement. The advantage of recording daily water use versus trying to record daily feed disappearance is that water meters are readily available and if water delivery devices are well-maintained, water will generally always be available to pigs.

Which changes in the pattern of daily water usage are the best predictor of pig health and performance is still unclear. Based on producer and veterinarian observations, when daily water usage drops for three continuous days, or drops more than 30% from day to day, this may indicate that a potential health challenge is occurring in the production facility. These changes in usage pattern should serve as an indication to the caregiver to look more closely at the pigs for signs of illness or discomfort. A spreadsheet to create barn sheets for the purpose of charting daily water patterns is available online at: *http://porkcentral.unl.edu.*

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When do pigs drink?

Water usage patterns in farrowing facilities do not show a distinct pattern within a 24-hour period. Milk let-down (lactation) occurs every 40 to 60 minutes so it is logical that the sow will consume water multiple times during the 24-hour day if water is continuously available (Figure 2). Sow's milk is primarily water, and milk yield generally increases until a peak at approximately three weeks post-farrowing. Daily water usage during lactation parallels this pattern (Figure 3).

Water consumption by nursery and grow-finish pigs has a distinct pattern within a 24-hour period. While there is very good evidence that a majority of water consumption is associated with eating activities in research settings, there are limited data on patterns of water usage in commercial facilities. Figures 4, 5 and 6 document the pattern of water use in wean-finish facilities at three locations in Nebraska and Minnesota. These facilities vary in the number of pigs per pen, the type of feeder and drinker, the type of ventilation, relative pig health, etc. The patterns were recorded over a seven-day period 4.5 to 5 months after weaning. The similarities between the winter and summer patterns at the three sites suggests two patterns of water usage exist, depending on the temperature in the facility (i.e. time of the year). In thermal-neutral conditions (generally air temperatures in the pig zone <80°F), grow-finish pigs begin drinking water around 5 to 6 a.m., with a peak in drinking water disappearance in early afternoon and a gradual decline the remainder of the day. This pattern is in agreement with published literature.

However, when pigs are growing in warm to hot conditions (air temperatures in the pen exceeding 80°F for one or more hours per day), they appear to alter their pattern of drinking water usage. Pigs begin drinking earlier in the day, with a morning peak from 8 to 9 a.m. There is a decline in drinking water use midday with a second peak in drinking water use from 5 to 8 p.m. followed by the decline into the night hours.

It is interesting to note that pigs shift to this pattern of drinking water use on the first day of air temperatures in the pig zone >80°F or so and maintain the pattern for three to five days, even if these subsequent days have temperatures considered to be thermal-neutral. This adaptation is often maintained for several days in anticipation that the heat stress event will be longer than a single day. This suggests that a shift in eating and drinking behavior is one of the first adaptations of the growing pig to heat stress. In the future, it may be possible to use this shift in drinking water usage as a predic-

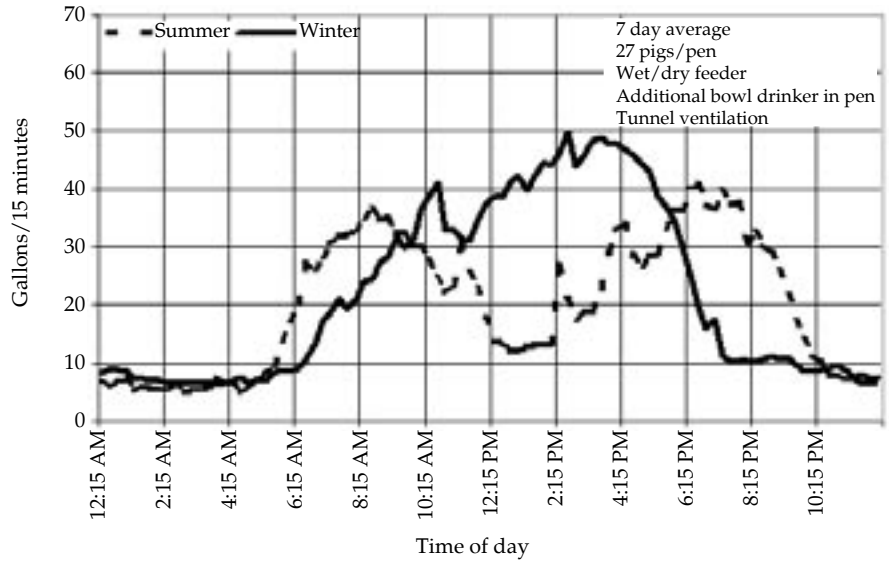

Figure 4. Effect of season on 24-hour water usage pattern in a 1200-head wean-finish facility five months after weaning in central Nebraska. Data courtesy Dicamusa.com.

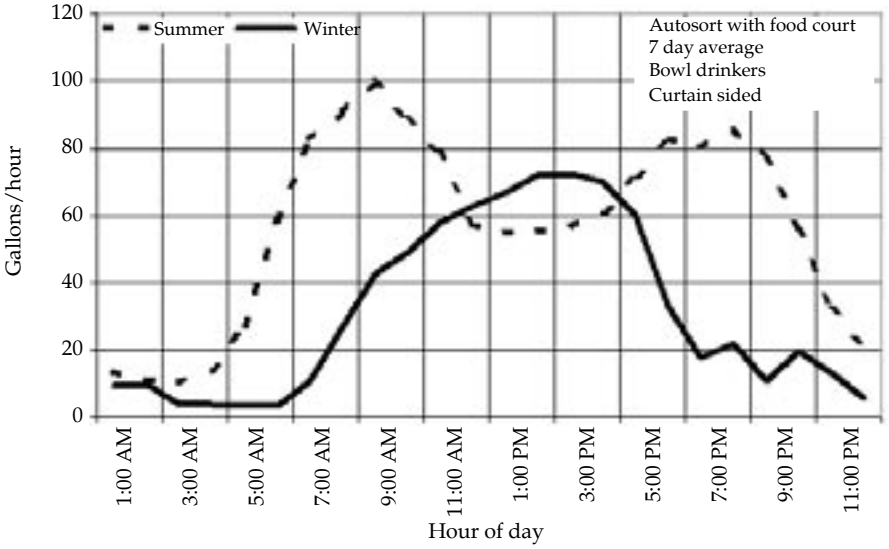

Figure 5. Effect of season on 24-hour water usage pattern in a 600-head fully slatted wean-finish facility in Southeast Minnesota when pigs averaged 195 to 210 lb body weight. Data courtesy Herdstar.com.

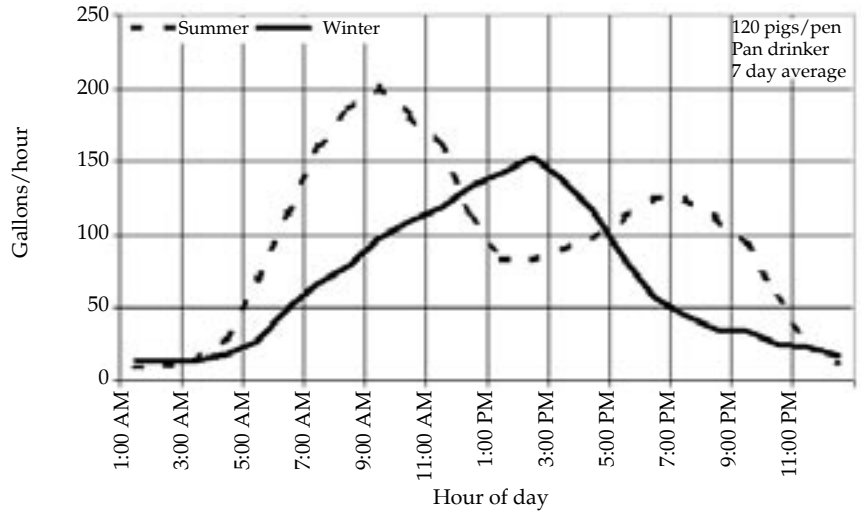

Figure 6. Effect of season on 24-hour water usage pattern in a wean-finish facility in eastern Nebraska 4.5 months after weaning. Data courtesy Dicamusa.com.

tor of a performance reduction due to heat stress in grow-finish pigs.

In addition to detecting of heat stress and potential disease outbreaks, automatic logging drinking water usage every 15 minutes has allowed for the detection of water leakage from drinkers in nursery and grow-finish facilities. That is, if drinking water usage is being logged every 15 minutes, there should be one or more 15-minute periods each day (generally midnight to 2 a.m.) when there is no water usage logged. If water usage is logged for every recording period, it is likely that one or more drinking devices are leaking, resulting in wasted water going into manure storage devices.

Conclusion

Knowledge of the daily water needs of pigs, and the patterns of water usage within the day, allow for the appropriate sizing of delivery devices and prediction of the impact of pork production on available water supplies. Daily charting of drinking water usage can serve as a predictor of the onset of swine health challenges such as swine influenza. As more sophisticated methods become available to record water usage, other predictors of performance may be developed depending on the patterns detected.

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Producers' Decisions

Allen Prosch[1]

Summary and Implications

The business decisions pork producers make are extremely important. Decisions increase in importance at the same time they become harder to make. In business management studies, time has been devoted to learn how such decisions can be made. Less study has been expended on how producers currently make decisions. In the United States, family producers have traditionally made decisions with information they could gather independently. The ability to create decision making information is difficult. Producers need to remember the key success item — that of effective management led by sound decisions. The process of decision making involves skills and abilities that can be learned. Attitudes towards risk and perceptions of agriculture have influenced producers to make decisions that do not reflect just the economics of the production sector. Also, off-farm employment and federal program payments have an effect on farm exits and on those exiting the pork enterprise, but who remain in farming. Changing the perceptions and attitudes of these producers may enable good producers to become more positive about their future in the industry.

Introduction

The business decisions pork producers make are extremely important. Decisions increase in importance, at the same time, they become harder to make. Producers face a number of challenges in their operations that are not directly related to their ability to produce pork.

One important change occurring in agricultural production is the change in business strategies. In business management studies, time has been devoted to learn how such decisions can be made. Fewer studies have been made on how producers currently make decisions.

In the U.S., family producers have traditionally made decisions with information they could gather independently. Producers would have been able to try several approaches to production in the past, but now the capital required, both monetary and physical, the risk involved and the margin to be gained, do not allow for many errors. However, the decision

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framework is becoming more complex. Producers need to deal with more information than ever. The ability to convert an overload of data into decision making information is difficult. And, the business environment is less tolerant of errors. Also, there is additional uncertainty risk and competition associated with changes in the agriculture and food industries. These increase the need for farmers to make informed decisions and have a plan for their businesses.

Decisions Now Involve More Pprocesses

Traditionally, an operation's resources were primarily labor, buildings and equipment. Today, an operation's resources may include intangible assets such as marketing systems, decision-making processes, coordinating systems, and established patterns of production. These systems often have high volume sales and purchases, professional expertise, skilled and motivated managers, alternate access to equity and debt capital, and sophisticated risk management practices that add to their competitiveness. Therefore, producers of all sizes are asking if they are large enough. But, producers need to remember the key success item — that of effective management led by sound decisions.

Decisions Involve More Skills and Abilities

The process of decision making involves skills and abilities that can be learned. However, that management ability, especially on smaller operations, does not improve without outside support. When managerial ability is fixed, decisions are made that do not reflect the true economies of the operation. Thus the size of an operation appears to be a driving factor in success, but not for production reasons as much as for management reasons.

Producers' decisions can be influenced by factors that have little or no relationship to the outcome of the decision. Most notable is the dramatic reduction in the number of pork producers who have decided to exit the industry in the past 15 years. Producers leave the industry despite having operations that are cost effective. From 1989 to 2002, producers with a 125-sow farrow to finish operation, with average production, would have had four years out of 14 in which that enterprise would not have generated all of an average family living. While the amount of funds generated by the swine enterprise for other farm expense or reinvestment went down, those producers who quit did so despite having successful swine enterprises.

Producers exit the industry for a variety of reasons, including production, economic, educational, environmental, and social issues, many of which are intangible. Producers who quit production from 1992 to 1996 were surveyed in 1997. Those producers surveyed gave low prices as the number one reason for leaving the industry. However, 82% of the respondents said they did not know their cost of production. Of the 18% who did know, the average cost was $39.03. This value would have been below the average Iowa / Southern Minnesota market prices in all the years included in the survey. However, only 10% of these producers had operations that would have 125 sows. They reported only 40% of their livelihood was provided by the swine enterprise. One-third of the producers increased another enterprise to use their time, but 45% reported working fewer hours on the farm. Seventy-five percent of the swine facilities used by these producers were reported as of types that by 1992 were becoming obsolete. And 73% of the producers indicated they planned to destroy the facilities, rather than use them for any purpose. While low prices may have been the stated cause for the action in the producers mind, the economic outcomes, contributory income, use of assets, and use of time do seem to be highly important. Often economic decisions are thought to be driven by maximizing economic return. That does not appear to be the driver here.

In a survey of producer's decision making conducted in 1992, producers indicated that marketing was a weak point in their operations. They identified help with marketing as a critical need. However, among numerous financial resources they might use, they indicated strongly they would not hire marketing help. In other financial areas of equal importance and need, they indicated they would or do hire help. It appears that producers of all commodities are concerned with low prices, but activities to help improve those prices on the marketing side are not well accepted.

Decisions Are Influenced by Attitudes

In a 2002 report on producers' decisions involving off-farm work, it was found that attitudes about risk influence the decision. As a result of risk aversion, a producer was likely to diversify income through off-farm labor endeavors. While farmers engaged in livestock production were more likely not to seek off-farm work, largely due to the constant on-site labor demand, the 1997 survey showed that once having quit the livestock operation, one-third of the respondents increased their off-farm work. It was also found that the greater the scale of production, the less likely the farmer would work off farm. Smaller pork producers who felt prices for hogs were inadequate may have chosen to exit the industry and take the option of off farm employment because it was seen as less risky.

In the mid-1990s, significant attention was given to forming networks of producers. It was thought that some of the value of larger systems could be captured by independent operations working together. In a 2002 report identifying independence as a decision influencing factor, it was found that even though the alternative may be profitable and less risky, not accounting for the value of independence would lead to underestimating the amount of profit necessary to attract farmers to such arrangements.

In a 2001 study of attitudes about profit and loss among another group of producers in an alternate farm enterprise, it was found that people tend to be about twice as upset about a loss as they would be happy about a gain of the same size. Looking back at low prices as the number one reason to exit the pork industry, this would support pork producers feeling much more discouraged by a few years of loss, despite numerous years of profit. Also contributing to this, in poor years the loss is often significantly larger than the yearly profit for better years. The dramatic difference has a greater impact on the attitude of producers than the actual economic reality. Producers also are affected by their attitude toward marketing tools used to improve prices. The combination of perceptions along with the attitude towards risk, affect the decision to participate in an enterprise.

A 2005 survey of producers involving the influence of weather and climate information showed the greatest improvement in use and influence of weather and climate forecasts will come from changing the individual's attitude. Again, an individual's perceptions of and attitudes about the information outweighed the application of useful information.

Final Thoughts

Producer decisions in the pork industry at the production level have been driven by factors other than economic return. As the industry has changed, diversified pork producers have responded to that change similar to other groups of farmer producers.

Attitudes towards risk and perceptions about the pork industry have influenced producers to make decisions that do not reflect just the economics of the production sector. Also, off-farm employment and federal program payments have an effect on farm exits and on those exiting the pork enterprise but remaining on the farm. These effects still exist. In a recent survey, 44% of producers still "feel" their future in the industry is severely threatened.

It is clear that many producers who are capable of competing in pork production feel threatened by change. Changing the perceptions and attitudes of these producers is a difficult task; however, doing so may enable good producers to become more positive about their future in the industry.

[1]Allen Prosch is the Pork Central coordinator at the University of Nebraska–Lincoln. References are available from the author by request.

Odor Footprint Tool Progress: Regional Output Resources

Richard R. Stowell
Dennis D. Schulte
Richard K. Koelsch
Christopher Henry[1]

Summary and Implications

This article highlights practical applications for resources being developed using the Odor Footprint Tool and the effects of differing regional weather patterns on needed setbacks by describing resources created for the regions surrounding Norfolk and Lincoln, Neb. The Odor Footprint Tool is being developed to help people assess the odor impact of new and expanded animal production facilities on the surrounding areas and use science-based information to establish minimum setback distances. Progress continues to be made toward development of a system that can be used in the field to develop site-specific odor footprints. As an intermediate step in this process, regional sets of Odor Footprint Tool resources are being developed for more general use. Odor roses, directional setback distance curves, and odor footprints are being produced for six regions in Nebraska. Odor roses provide a descriptive picture of the directionality of odor annoyance within a region, independent of the type or size of livestock facility involved. Odor roses are well suited for general planning and educational purposes where mainly the directional fate of odor emissions is desired. Directional setback distance curves facilitate determining minimum setback distances in four 90-degree sectors around a site, based upon the total odor emission rate of the site. The total emission rate depends on the size and type of livestock housing and/or manure storage facilities involved, and whether any odor control technologies are

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implemented. Directional setback distance curves are especially useful when principal setback distances are desired, and when a number of preliminary comparisons are to be made. Odor footprints show curves similar to contour lines representing the locations around a livestock site that have common expected frequencies of odor annoyance. Odor footprints correspond to specific scenarios (having specific total odor emission rates) and are useful for visualizing the projected odor impact of an operation on the surrounding area. As livestock producers, their service providers, and regulatory officials begin to use these resources, they should be better able to make reasonable decisions regarding the odor impact of livestock operations on surrounding neighbors and rural communities. Odor impact at a given location is presented in terms of the likelihood that odor will exist at annoying intensity levels. Producers can use the frequency of annoyance information and the corresponding percentages of time that odor annoyance is not expected (odor annoyance-free frequencies) to help evaluate their risk of offending neighbors and to determine which neighbors are at greatest risk. This information will be helpful when evaluating sites and in determining the benefit of implementing proven odor control technologies. Also, regulatory officials will have access to science-based information that can form the basis of reasonable discussions at public hearings and be considered in decision processes for applications to build livestock facilities.

Background

As livestock and poultry producers have expanded and intensified their operations, the level of community concern and number of complaints registered about emissions of air pollutants, especially odorants, from animal production facilities have risen dramatically as well. One approach to deal with these concerns involves establishing minimum setback (separation) distances between production facilities and residences or public facilities. Many county governing bodies have implemented setback requirements through local zoning regulations, and most of these lack a sound scientific basis.

Current siting requirements for new livestock and poultry production systems in the United States are based mainly on the number and weight of animals on a site and the distance to the nearest neighbor. This approach does not account for existing odor sources in a community, the influence of localized meteorological or topographic factors on odor dispersion, or the use of improved odor management practices. Odor dispersion is a complex process that depends on emissions characteristics of the source, weather patterns, terrain, and the presence of other odor sources.

Atmospheric dispersion models can account for these factors and could provide rural communities and the livestock industry with the tools needed to incorporate science and objectivity into the odor management decision-making process. Air quality research groups at the University of Nebraska and the University of Minnesota developed the Odor Footprint Tool for estimating setback distances. The Odor Footprint Tool uses an EPA regulatory model (AERMOD), which was selected because it has considerable flexibility, and the regulatory community generally accepts its use. The Odor Footprint Tool uses meteorological data from sources such as the National Weather Service (NWS) and the Automated Weather Data Network (AWDN), which has numerous weather stations located throughout Nebraska. An interface was also developed to collect necessary information from the user and process it for use by AERMOD. The Odor Footprint Tool can then use the AERMOD output to generate *odor roses, directional setback distance curves,* and *odor footprints.* The Odor Footprint Tool has gone through an initial calibration stage to facilitate accurate prediction of odor intensities downwind of an odor source. Validation of the Odor Footprint Tool for use with a swine finishing facility in a community setting is underway in Nebraska.

Although the Odor Footprint Tool is being developed to handle more varied and specific situations, the focus of much of the effort to this point has been on producing output resources for generic situations within regions surrounding readily identified primary weather stations. Output resources are being developed for six regions encompassing the state of Nebraska (see Figure 1), three regions in South Dakota, and a region each within Iowa, Kansas, and Minnesota. These regional resources are being developed for educational purposes and in preliminary planning of livestock facilities — applications where local terrain and proximity to the regional weather station are not generally critical. This article highlights practical applications for the regional resources being developed using the Odor Footprint Tool and the effects of differing regional weather patterns on needed setbacks by describing resources created for the regions surrounding Norfolk and Lincoln, Neb.

Description of Output Resources

All of the information presented is based upon historical weather conditions from April 15 through October 15, sometimes referred to as the "odor season" in the Midwest. People are more likely to be exposed to odors during this period since the warm months of the year are generally when odors are most prevalent and people are active outdoors. The term "odor annoyance" corresponds to an intensity of 2 or higher on a 0-to-5 n-butanol scale as assessed by trained individuals. The term "odor unit," the value of which is

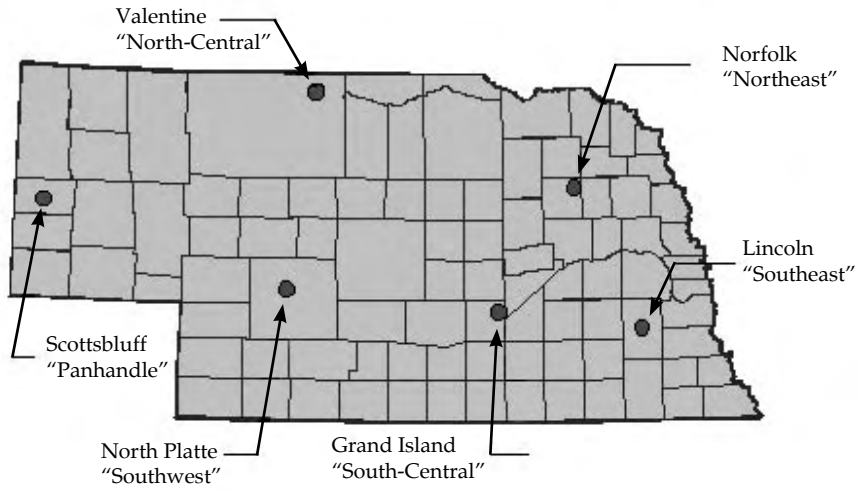

Figure 1. Weather station locations for the six Nebraska regions for which Odor Footprint Tool output resources are being developed.

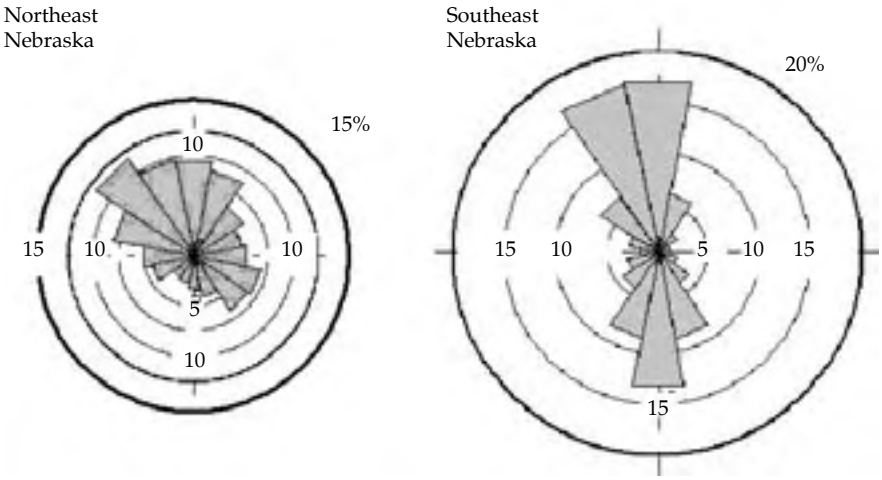

Figure 2. Odor roses for Norfolk (left) and Lincoln, Neb. (right). The extent of the radial bars represents the proportion of total annoying odors expected in that direction.

assigned by a trained odor panel, is used to quantify odor concentration and the rate at which odor is being emitted from a facility.

Odor Roses

An odor rose (Figure 2) shows the likelihood of annoying odors existing in a given direction from a livestock facility, independent of the size or type of operation. The likelihood of annoyance is expressed as the percentage of the total annoyance incidences for all directions, so the sum of the sector bars in all directions equals 100%. For example, the comparative likelihood of annoying odors existing directly to the south of an odor source is about 3% near Norfolk versus 13% near Lincoln.

The likelihood of being exposed to annoying odors is a function of both surface and upper air weather conditions in the region over an extended period of time (typically 10 years). Wind direction logically plays a key role in the directionality of odor annoyance. Influences of other factors such as humidity, cloud cover, and atmospheric stability also are evident, however, and the odor roses that have been developed are not mirror images of the corresponding wind roses for the given locations.

Near Norfolk, odor annoyance is likely to be most prevalent to the north of a source, with maximum odor annoyance to the northwest (Figure 2). In contrast, odor annoyance near Lincoln is expected to be very polarized with maximum annoyance to the north and north-northwest of an odor source followed closely by the due south direction. These differences in weather patterns have noteworthy implications for planning and assessing sites for livestock facilities in the two regions.

As a point of interest, each of the directional bars within the odor roses has a small, darkly shaded interior sector while the outer portion is lightly shaded. The interior sectors represent expected odor annoyance during daytime hours (8:00 a.m. to 6:00 p.m.), and the outer portion represents nighttime and transition hours. It is quite apparent from the odor roses shown that the potential for annoying odors is greatest during transition and nighttime hours, when the atmosphere is more likely to be stable. Near Norfolk, the total likelihood of annoying odors existing between 6:00 p.m. and 8:00 a.m. (a 14-hour period or 58% of a day) is about 86%, while between 8:00 a.m. and 6:00 p.m. it is only 14%. For the Lincoln area, these percentages are 88% and 12%, respectively. Therefore, the directional nature of odor annoyance for the transition and nighttime portions of a day is representative of the full day.

Directional Setback Distance Curves

Directional setback distance curves are used to determine minimum setback distances in the four principal directions downwind from an existing or proposed livestock facility. Directional setback

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distance curves were developed based upon the concepts presented with OFFSET, a groundbreaking setback-estimation tool developed at the University of Minnesota. Using a worksheet and graphs that apply for the geographic region in which the facilities are to be located, four directional setback distances can be determined for a specified odor-annoyance-free frequency. Each of the four distances represents the minimum setback desired for a corresponding 90-degree sector extending to either the north, south, east, or west of the site; or, alternatively, to the northeast, southeast, southwest or northwest. The alignment of the directions for a given region was selected to match the direction of maximum expected odor impact with one of the 90-degree sectors. For example, the odor roses shown in Figure 2 show that the maximum odor impact of a generic odor source near Norfolk would be expected to the northwest, while for the Lincoln area, the maximum projected impact would be more due north of the facility. Therefore, directional setback curves were developed for each of these two regions (Figure 3), but each set of curves is based on a different axis to highlight the direction of maximum odor impact.

Each set of curves shows curves for 90%, 94%, 96%, 98% and 99% odor-annoyance-free frequencies. The percentage values represent the minimum proportions of hours during the spring-through-fall period, during which a residence situated at or beyond the setback distance would not be exposed to annoying levels of odor coming from the livestock site. In other words, using the 96% curve, odors at locations inside the identified setback may be present at annoying levels more than 4% (100% - 96%) of the time, while odors at locations outside the setback would be expected to be present at annoying levels less than 4% of the time. The

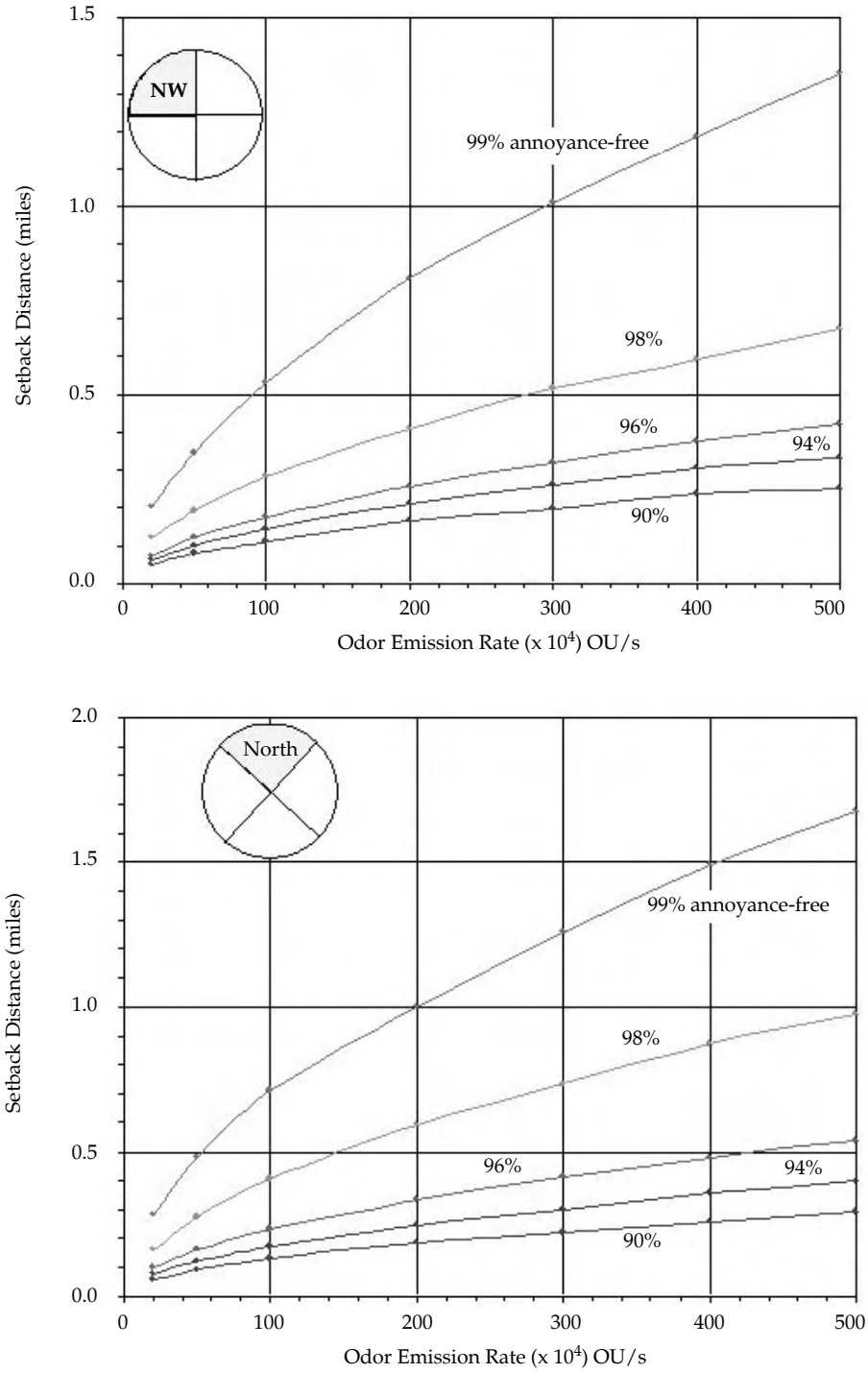

Figure 3. Directional setback distance curves for regions surrounding Norfolk (top) and Lincoln (bottom), Neb. Graphs shown are for the direction of maximum projected odor impact. Graphs showing curves for the other three primary directions are available but not shown.

listed percentages were selected as covering the practical range of acceptable odor annoyance, representing from two to 18 full days of odor annoyance every year from mid-April to mid-November. The separation distance required to achieve a greater odor-annoyance-free percentage increases significantly with each percentage point increase. For example, the difference between the setbacks for 98% and 99% odor-annoyance-free frequencies is at least twice that needed to move from 90% to 94%. Therefore, lower tolerance for risk of exposure to annoying odors is directly reflected by noticeably larger required separation between the source and receptor. Note that it is not possible to determine a setback distance for 100% odor-annoyance-free conditions.

The setback distances described by these curves take into consideration historical weather conditions that influence odor transport and dispersion in the selected region. If the influence of terrain and local weather conditions are required to obtain a more accurate determination of setbacks, then a site-specific footprint should be produced.

The setback distance for a livestock facility within a given region is determined based upon the total scaled odor emission rate from all noteworthy odor sources on the site – as shown along the horizontal axis of the graph. Scaled odor emission rates (OER) for individual facilities are found using the following formula:

> OER = Odor emission number x Plan area x Odor control factor

Two pieces of information about the facilities on a site are required to estimate directional setback distances: the types of [proposed] facilities on the site and each facility's floor or surface area. Most general types of facilities considered will have an odor emission number associated with them. The odor emission number represents the relative amount of odor one could expect to be released by the source facility into the surrounding air per unit of floor or surface area. These values are based upon currently available emissions data and as more data becomes available, these values may be updated. The odor emission numbers are scaled for use with AERMOD and are for use with the Odor Footprint Tool only.

An odor control factor (value between 0 and 1) also may be applied to assess the impact of using odor control technologies. The more odor reduction provided, the lower the odor control factor. Several odor control technologies have been evaluated sufficiently to determine their effectiveness in reducing odor emissions and assign appropriate odor control factors.

Using the appropriate set of directional setback distance curves, a calculated total odor emission rate, and a selected odor-annoyance-free frequency, one can read off the minimum setback distance for each of the four primary directions around the site. Information on odor emission numbers and odor control factors will be provided separately as it becomes available, along with a worksheet to use in making calculations and recording setback distances.

To illustrate the use of these curves, consider a swine finishing building housing 2,000 hogs and having slatted flooring over a deep pit. Assuming rough building dimensions of 45 ft x 400 ft (or 80 ft x 220 ft), the building has about 18,000 sq ft of floor area. Given that the odor emission number assigned this type of facility is 165 odor units (OU) per second per sq ft, the OER for the building is about 3,000,000 or 300 x 10^4 OU/s. Using Figure 3, the setback distance in the direction of maximum projected impact would be just over half a mile for a site near Norfolk and about 3/4 of a mile near Lincoln at 98% odor-annoyance-free frequency (fairly low tolerance for odor). These distances would jump to nearly 1 mile and 1.3 miles, respectively, at 99%. By employing additional odor control, one could reduce the odor impact of the complex and the setback needed. For example, spraying [vegetable-based] oil inside the pig space to control dust has been demonstrated to reduce odor emissions by about 50%, so the total OER of this complex could drop to about 150 x 10^4 OU/s [18,000 x 165 x 0.5 ~ 1,500,000] with oil sprinkling, and the setback distance at 98% would now be about 0.3 miles in the northwest direction near Norfolk and 0.5 miles to the north near Lincoln.

Odor Footprints

An odor footprint shows a plan [top] view of the projected odor impact of a livestock operation in terms of the extent of exposure to annoying odor in all directions from the source (Figure 4). Using the concept of contour lines, curves are plotted showing the locations of constant odor-annoyance-free frequency (100% minus the frequency of annoyance).

Odor footprints are tied to a specific odor emission rate, which was described in the previous section as a function of the number, types and sizes of facilities on a site, and whether any odor control technologies are implemented. Figure 4 contrasts odor footprints for the regions surrounding Norfolk and Lincoln, respectively, for facilities having a total odor emission rate of 500 x 10^4 OU/s. For illustrative purposes, the scaled emission rate from a 3,300-head swine finishing building with deep pits and no special odor control practice in place is about 500 x 10^4 OU/s. Note that the same total odor emission rate could be achieved for numerous combinations of facility types and sizes, or through the use

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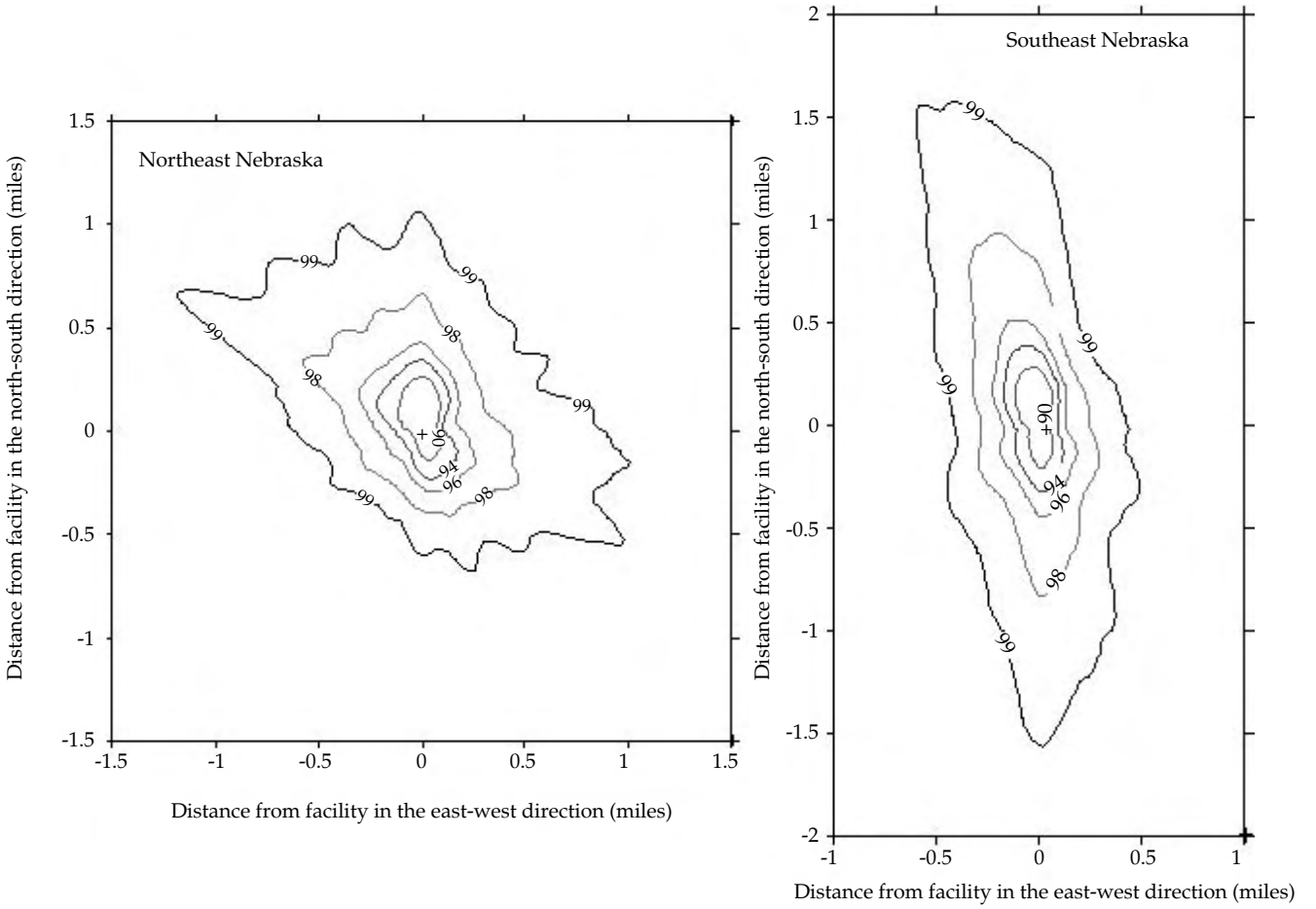

Figure 4. Odor footprints for Norfolk (left) and Lincoln, Neb. (right) at total odor emission rates of 500 x 10^4 OU/s. Curves show locations with common odor-annoyance-free frequencies.

of odor control on larger facilities. For example, a 3,300-head finisher with a shallow pit and lagoon would most likely have a different odor emission rate, as would a 3,300-sow gestation barn.

An immediate observation that can be made is that the shapes of the footprints in Figure 4 differ for the two regions, with each corresponding to the basic shape of the odor rose for that region. Looking at the detail of each footprint, both have five closed loops plotted representing locations having odor-annoyance-free frequencies of 90 to 99%. As the distance from the source increases, less odor annoyance should occur as indicated by greater odor-annoyance-free frequencies.

Both the extent of projected odor impact and the directions of maximum and minimum impacts differ noticeably for the two regions (Table 1). These differences, along with the fact that neither footprint shows a circular odor pattern around the source, highlight the deficiencies of employing a constant setback scheme or bulls-eye approach to account for odor. For the region surrounding Lincoln (southeast Nebraska), the practical outcome of using a constant setback distance would be having an excessively conservative setback requirement to the east and west of a source facility and potentially having insufficient or a nonconservative setback to the north and south of the facility.

Regional footprints do not consider the effects of local terrain, nor are these footprints necessarily based upon surface climatic data that are applicable for all locations within a given region. Enhancements to the Odor Footprint Tool will facilitate the development of site-specific odor footprints that can be used by consultants and technical service providers with individual operations for in-depth planning purposes.

Summary and Conclusions

The Odor Footprint Tool, which uses the AERMOD dispersion-modeling package, was used to develop regional resources for assessing odor impact from livestock and poultry operations. Three output resources — odor roses, directional setback distance curves, and odor footprints — were described, along with their respective practical applications.

Table 1. Sa
of 500 x 10^4 OU/s.

Odor-annoyance-free frequency	Norfolk (Northeast Nebraska)		Lincoln (Southeast Nebraska)	
	Smallest setback distance	Largest setback distance	Smallest setback distance	Largest setback distance
	Direction = SW	Direction = NW*	Direction = East	Direction = NNW
90%	300	1,200	300	1,200
98%	1,600	3,400	1,200	4,700
99%	2,200	7,100	2,200	8,700

*For 90%, the maximum separation distance is to the north of the source.

The odor rose offers basic insights into a region's directional risk for odor annoyance, independent of the nature of a source. Directional setback distance curves can be used to determine minimum setback distances in principal directions around a facility. Comparing of alternative sizes of operations, odor control options, tolerance levels for odor, etc. can readily be performed using these curves. Odor footprints can be developed for specific facility and odor control scenarios. Odor footprints are effective resources for visualizing the potential impact of a livestock odor source on the surrounding area. These regional resources will be made available to producers and other interested parties on appropriate Web sites and as extension materials.

Acknowledgement

Financial support from the National Pork Board was provided for this project.

[1]Richard R. Stowell is an assistant professor, Dennis R. Schulte a professor, Richard K. Koelsch an associate professor, and Christopher Henry an extension engineer in the Department of Biological Systems Engineering at the University of Nebraska–Lincoln.

Freezing Swine Embryos: Do Success Rates Differ Between Breeds?

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Summary and Implications

Successful freezing, or cryopreservation, of embryos could greatly impact the pork industry, serving as a tool for conservation of valuable germplasm and enhancing biosecurity for transfer of genetic material. Pig embryos are very sensitive to cooling and few reports have shown successful developmental rates following freezing. The objectives of this study were to determine the efficiency of freezing pig embryos using a microdroplet vitrification method and to investigate in vitro development of embryos from Chinese Meishan and occidental white crossbred females following cryopreservation at different stages of embryonic development. Preliminary studies using the microdroplet vitrification method for cryopreservation and embryo transfer into recipient females resulted in the birth of normal, live piglets indicating the effectiveness of this procedure. Rates of expanded blastocyst formation did not differ between Meishan and white crossbred nonfrozen, control embryos (98 and 95%, respectively). Developmental rates were significantly higher for control embryos than vitrified embryos from both Meishan and white crossbred females at the expanded blastocyst stage ($P < 0.001$), but not at the hatched blastocyst stage. Following collection of embryos from Meishan and white crossbred females, cryopreservation and in vitro culture, the percentage of cryopreserved embryos alive after 24 hours of culture was higher for Meishan (72%) than white crossbred (44%; $P < 0.001$) embryos. However, development of thawed, cryopreserved embryos that survived 24 hours of culture was not different for Meishan and white crossbred embryos at the expanded (64%) or hatched (22%) blastocyst stages. The optimal stages to vitrify pig embryos using the microdroplet method range from late compact morula to early expanded blastocyst. Our results suggest that Meishan embryos have a higher capacity to survive the freezing process than white crossbred embryos, independent of embryo stage.

Background and Introduction

There are approximately 940 million swine in the world today and a large portion of the human population includes pork as an

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important source of protein in the diet. Cryopreservation of porcine embryos could greatly impact the pork industry by serving as a tool for conservation of valuable germplasm and enhancing biosecurity for transfer of genetic material. However, pig embryos are very sensitive to cooling and ice crystallization during the freezing process. Therefore, the efficiency of cryopreservation is much lower in pig embryos than in embryos from other species.

To avoid ice crystal formation in the freezing medium, the vitrification method appears to be the most promising technique for cryopreservation of swine embryos. During vitrification, a type of glass is formed in the freezing medium preventing embryos from being subjected to cellular damage associated with ice crystal formation. Porcine embryos have been successfully frozen at the hatching and hatched blastocyst stages. At these stages however, the zona pellucida, a protective coating surrounding the embryo that is similar in function to the shell of a chicken egg, is unable to act as a barrier against infectious organisms. Cryopreservation also has been successful with embryos from the 4-cell to early blastocyst stages. However, the cryopreservation procedure used in those studies requires creating a hole in the zona pellucida to remove the lipid content after centrifugation. This manipulation disrupts the intact zona pellucida, increasing the susceptibility of embryos to disease transmission.

Transfer of zona-intact embryos that were frozen using the open pulled straw (OPS) method and in combination with cytoskeletal stabilization prior to vitrification, produced live piglets. Scientists at the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) in Beltsville, Md. reported that the OPS method improved the survival rate of pig embryos vitrified prior to hatching from the zona pellucida. In addition, other investigators have shown that it is possible to freeze porcine embryos at the compact morula stage and produce live offspring using the OPS freezing method without cytoskeletal stabilization pretreatment. Using a modified microdroplet method, described previously to vitrify bovine oocytes, researchers at the National Livestock Breeding Center in Japan successfully produced piglets from vitrified compact morula and early blastocyst stage embryos. In this study, the pregnancy rate of embryo transfer recipients was 40% and the percentage of live piglets born per embryo transferred was 10%. The advantage of this method is that it does not require chemical pretreatment or manipulation of the zona-intact embryos.

There is much interest in studying reproductive differences between Chinese Meishan and occidental breeds of swine. Meishan females are more prolific than females from white crossbred lines (four to five more pigs per litter). Factors that contribute to the increased litter sizes of Meishan females include ovulation rate, embryonic survival and uterine capacity. Investigators from France reported that Meishan embryos could withstand the vitrification process better than embryos from hyperprolific Large White females. However, the mechanisms underlying this difference have not been investigated. The comparison of embryonic development after cryopreservation between embryos from different genotypes can aid in the discovery of important factors for embryonic survival after vitrification. Thus, the objectives of this study were: 1) to determine the efficiency of freezing pig embryos using a microdroplet vitrification method, 2) to examine *in vitro* development of Meishan and white crossbred embryos following cryopreservation, and 3) to determine the importance of embryonic stage for Meishan and white crossbred embryos on survival rates after cryopreservation by microdroplet vitrification method.

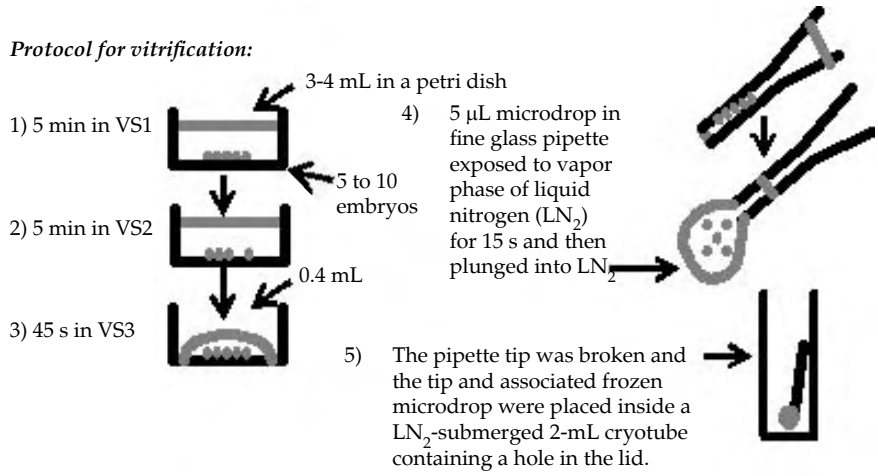
Materials and Methods

Efficiency of Microdroplet Method

The microdroplet vitrification procedure was used with some modifications from the original published protocol described by scientists at the National Livestock Breeding Center in Japan. Embryos from white crossbred females on day 5 following estrus (day 0 = onset of estrus) were flushed from donor reproductive tracts at surgery using Beltsville Embryo Culture Medium (BECM). After flushing, the embryos were selected, cryopreserved by the microdroplet protocol, and stored in liquid nitrogen (-196°C) for approximately one hour (Figure 1). Immediately prior to embryo transfer, the embryos were thawed using a four-step procedure (Figure 1). Two white crossbred recipients on day 5 of the estrous cycle received either 24 compact morulae or 24 compact morulae/blastocysts. The recipients were checked for return to estrus over 30 days and allowed to gestate until farrowing.

Comparison of Development between Meishan and White Crossbred Embryos Following Cryopreservation

Embryo collections were performed at the USDA, ARS, Roman L. Hruska U.S. Meat Animal Research Center in Clay Center, Neb. and transferred for four hours at 39°C to the Department of Animal Science at the University of Nebraska–Lincoln. First parity Meishan sows (n = 11) and white crossbred gilts (n = 13) were observed for estrus every 12 hours and naturally mated at 12 and 24 hours after the onset of estrus within breed using two different boars. Females were slaughtered between day 4.5 and 6 after estrus and embryos were collected. Compact morula and blastocyst stage



VS1: BECM + 5% EG + 0.57 M sucrose
VS2: BECM + 2.5% EG + 0.27 M sucrose + 1% polyethylene glycol (PEG)
VS3: BECM + 40% EG + 0.36 M sucrose + 2% PEG

Protocol for thawing:

1) 5 min in WS1 → 2) 5 min in WS2 → 3) 5 min in WS3 → 4) 5 min in WS4

WS1: BECM + 5% EG + 0.57 M sucrose
WS2: BECM + 2.5% EG + 0.29 M sucrose
WS3: BECM + 0.3 M sucrose
WS4: BECM

Figure 1. Vitrification and thawing aspects of the microdroplet protocol for cryopreservation of pig embryos. VS = vitrification solution. WS = warming solution. BECM = Beltsville embryo culture medium.

embryos from each female within breed were randomly allocated either directly into the culture system to serve as controls (68 Meishan and 48 white crossbred embryos) or to undergo vitrification, storage in liquid nitrogen (-196°C) for one hour, thawing, and placement into culture (101 Meishan and 78 white crossbred embryos). Embryos from each treatment were cultured in 50 µl drops of modified Whitten's medium + 1.5% BSA under oil at 37°C in a 5% CO_2 in air environment and scored for development at 24, 48 and 72 hours of culture. Embryos were considered to have survived if they advanced a stage in development following 24 hours of culture and did not show signs of lyses or degeneration. The percentages of expanded and hatched blastocysts were calculated based on the number of surviving embryos only.

Importance of Embryonic Stage on Survival Rates after Vitrification

A total of 93 blastocysts/early expanded blastocysts from four Meishan (n = 42) and seven white crossbred (n = 51) females were compared with 56 compacted 8-cells/early morulae from four Meishan (n = 26) and four white crossbred (n = 30) females to determine survival rates following cryopreservation. Following the thawing procedure, embryos were cultured for 24 hours as described above and survival rates were determined.

Statistical Analysis

Data were analyzed with a non-parametric X^2 test using the CATMOD procedure of the Statistical Analysis System. The percentages at different stages were compared between groups and were considered statistically significant if $P < 0.05$.

Results

Efficiency of Microdroplet Method

We performed two embryo transfers, placing 24 embryos into the uterine horn of each white crossbred recipient on day 5 of the estrous cycle. On day 21 of gestation, the female that received 24 compact morulae returned to estrus. The recipient that received a combination of compact morulae/blastocysts (n = 24) produced six live offspring. These piglets were healthy and exhibited a normal phenotype (See cover picture).

Comparison of Development between Meishan and White Crossbred Embryos Following Cryopreservation

The retrieval rates from the cryovials for both breeds were above 92% (Figure 2). The survival rate was higher for Meishan (72%) than white crossbred (44%; $P < 0.001$; Figure 2) embryos. However, *in vitro* developmental rates of embryos that initially survived cryopreservation to the expanded (64%) or hatched (22%) blastocyst stages were not different between breeds (Figure 3). Developmental rates to the expanded blastocyst stage were higher for control embryos than frozen embryos from both breeds ($P < 0.001$), but no breed difference was observed for development to the hatched blastocyst stage (Figure 3). Rates of expanded blastocyst formation did not differ between Meishan and white crossbred control embryos (98 and 95%, respectively), but more Meishan control embryos developed to the hatched blastocyst stage (22% for Meishan vs. 9% for white crossbred; $P < 0.05$).

Importance of Embryonic Stage on Survival Rates after Vitrification

The survival rate was much higher for embryos of both breeds

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vitrified at the blastocyst/early expanded blastocyst stage (74% for Meishan and 47% for white crossbred) than at the compacted 8-cell/early morula stages (31% for Meishan and 4% for white crossbred; $P < 0.001$; Figure 4). Once more, a lower tolerance to vitrification was observed for white crossbred embryos than Meishan embryos, independent of the initial embryonic stage ($P < 0.001$). Cryopreservation decreased expanded blastocyst formation equally for both breeds (Figure 4).

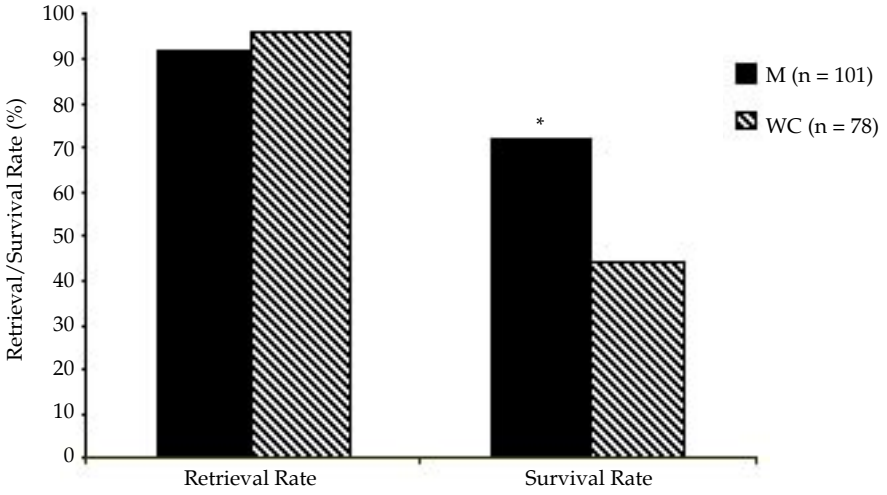

Figure 2. Comparison of embryo retrieval from cryovials (retrieval rate) and survival rate following cryopreservation of Meishan (M) and white crossbred (WC) embryos. Embryos were considered to have survived if they advanced a stage in development following 24 hours of culture. * $P < .001$ vs. WC.

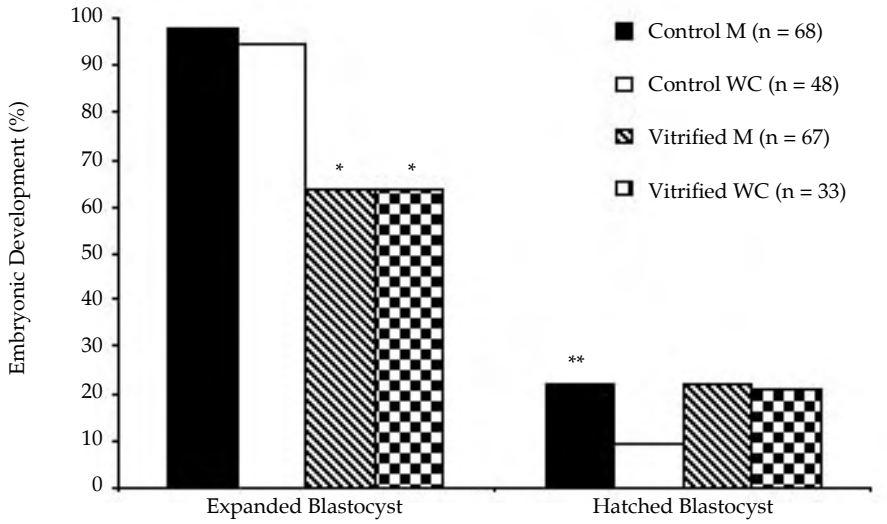

Figure 3. Effect of cryopreservation on development of Meishan (M) and white crossbred (WC) embryos *in vitro*. * $P < .05$ vs. controls. The only breed effect detected was for rates of hatched blastocyst formation in the control treatment. ** $P < .05$ vs. WC.

Discussion

Numerous attempts to freeze pig embryos in the past 30 years have resulted in low success rates, mainly due to the sensitivity of porcine embryos to hypothermia. In the 1990s and the beginning of this decade, several experiments were conducted resulting in protocols that permitted the birth of live and normal piglets from cryopreserved embryos. However, to perform these protocols with satisfactory results embryos must be exposed to cytoskeletal stabilization agents and/or micromanipulation procedures. Our studies suggest that it is possible to produce piglets after vitrification of zona pellucida-intact embryos without cytoskeletal stabilization agents and expands a previous report using a microdroplet protocol. The success of the OPS and microdroplet methods can be attributed to embryos passing through a critical temperature zone more rapidly than with conventional methods.

In several cases of porcine embryo vitrification, the type and concentration of permeable cryoprotectants have varied greatly. Cryoprotectants, such as ethylene glycol, can be highly toxic to embryos. Differences in cytoskeleton makeup may contribute to substantial species variation in sensitivity to cryoprotectants. The deleterious effects can include disruption of microfilaments during equilibration and after thawing can be evidenced as cell lyses and disaggregation, as well as nuclear and plasma membrane damage. A concern with the microdroplet protocol was the amount of time that embryos would be exposed to ethylene glycol. At higher temperatures, bovine embryos are susceptible to ethylene glycol when they are exposed to increased concentrations for extended periods of time (>10% ethylene glycol for over five minutes). The microdroplet protocol includes exposure times of 10 minutes in 10% of ethylene glycol and 45 seconds in the vitrification media containing 40% ethylene glycol, all at 37°C. During the thawing procedure embryos are exposed for more than 10 minutes in 2.5 to 5% ethylene glycol. The birth of live piglets described in this study and by investigators at the National Livestock Breeding Center in Japan suggests that pig embryos have a higher tolerance to ethylene

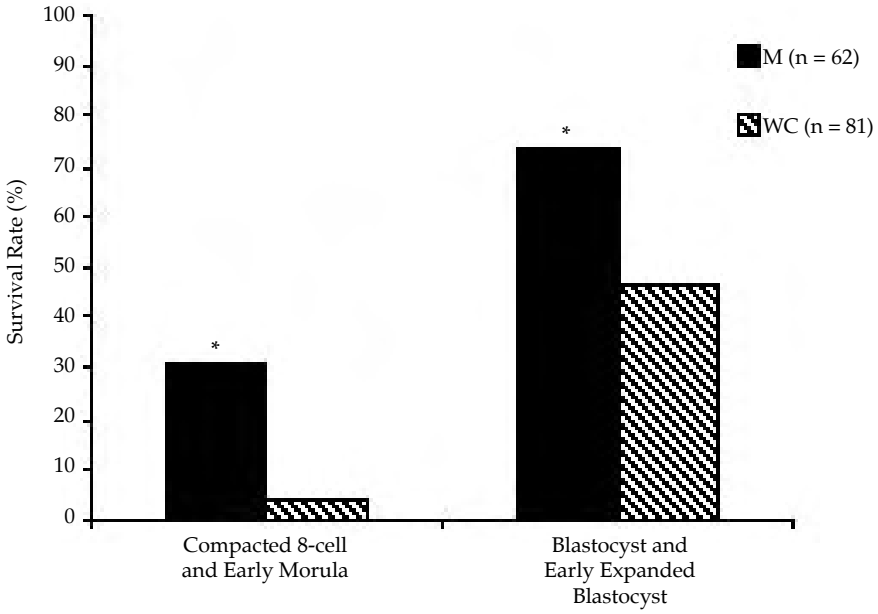

Figure 4. Effect of initial embryonic stage on survival rates of Meishan (M) and white crossbred (WC) embryos after cryopreservation. Embryos were considered to have survived if they advanced a stage in development following 24 hours of culture. Survival rates of all embryos were much higher for blastocyst/early expanded blastocyst than compacted 8-cell/early morula stages, regardless of breed ($P < .01$). The survival rate for M embryos was higher than WC embryos regardless of initial embryonic stage. * $P < .001$ vs. WC.

glycol than bovine embryos. We speculate that this phenomenon is due to a higher amount of lipid in pig compared to bovine embryos at the morula and blastocyst stages.

Only one French study has compared cryopreservation of embryos from females of Meishan and occidental breeds. These scientists, comparing embryos from Large White hyperprolific and Meishan breeds, found that Large White hyperprolific blastocysts (27%) had a lower viability *in vitro* than Meishan blastocysts (67%), when embryos were vitrified with phosphate-buffered saline. However, no difference between breeds (41 and 43%) was detected using Tissue Culture Medium 199 as the base vitrification solution. In the same study, developmental rates of vitrified morulae did not differ for the two breeds (11% for Large White hyperprolific and 14% for Meishan, respectively), although viability rates were low. In contrast, in the present study Meishan embryos survived the vitrification process better than white crossbred embryos at all developmental stages examined; compacted 8-cell, early morula, compact morula, blastocyst or expanded blastocyst. The difference between breeds for embryonic survival was almost 30%, regardless of developmental stage at cryopreservation. However, no difference was observed between breeds for *in vitro* development of embryos that initially survived vitrification to the expanded or hatched blastocyst stages. This finding is intriguing and indicates a unique mechanism present in Meishan embryos compared to embryos from white crossbred females.

The development of an efficient protocol to cryopreserve 8-cell and early morula embryos is important for the practical use of porcine embryo transfer in the field. Cryopreservation at these stages is possible, but these protocols involve micromanipulation, centrifugation and cytoskeletal stabilization. In the present study, the microdroplet method produced unsatisfactory results for survival rate of cryopreserved 8-cell compacted/early morula. The initial stage of the embryo had a strong effect on embryonic survival for both breeds. Approximately 40% more embryos survived when vitrified at the blastocyst/expanded blastocyst stages than at the compacted 8-cell/early morula stages. Consistent with this, porcine morulae are more sensitive to vitrification than blastocysts using the OPS method. It is important to point out that 8-cell pig embryos can show signs of compaction. These embryos can be easily confused with morulae or compact morulae. Therefore, researchers and technicians in the field must diligently evaluate embryos prior to vitrification. An incorrect evaluation could result in decreased survival rates of cryopreserved embryos.

Conclusion

Our study describes a vitrification method for zona pellucida-intact swine embryos that is effective in producing normal, live piglets. The optimal stage to vitrify pig embryos using the microdroplet protocol is at the blastocyst/expanded blastocyst stage. Further, our results suggest that Chinese Meishan embryos have a higher capacity to survive the vitrification process than white crossbred embryos. However, embryos from both breeds that initially survive vitrification have similar developmental capabilities *in vitro*. Improvements in embryo freezing procedures are likely to become increasingly important to the swine industry, especially with the advancement of nonsurgical embryo transfer in swine.

[1]Marcelo M. Montagner was a visiting scholar in the Animal Science Department at the University of Nebraska–Lincoln and recently received his doctorate from the Federal University of Santa Maria in Santa Maria, Brazil. Paulo B.D. Gonçalves is a Professor in the Biotechnology and Animal Reproduction Laboratory at the Federal University of Santa Maria. Ronald K. Christenson is a Research Physiologist at the USDA, ARS, Roman L. Hruska U.S. Meat Animal Research Center in Clay Center, NE. Ginger A. Mills is an Agricultural Research Technician and Brett R. White is an Assistant Professor in the Animal Science Department at UNL.

Regulation of Pituitary Gene Expression in Lines of Swine with Different Ovulation Rates

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Ginger A. Mills
Brett R. White[1]

Summary and Implications

Litter size plays a major role in the economics of pork production. Even modest increases in average litter size can have considerable effects on overall profitability. Two major components of litter size – ovulation rate and embryonic survival – have been used in a selection index project ongoing for several generations at the University of Nebraska–Lincoln (UNL). Additionally, the Chinese Meishan breed is one of the most prolific breeds, producing four to five more pigs per litter than white crossbred females. We investigated the role of the gonadotropin-releasing hormone (GnRH) receptor and gonadotropin subunit genes in determination of ovulation rate between lines of swine. Ten UNL Index and Control line white crossbred gilts and 12 Meishan gilts were ovariectomized following three (Index and Control) or 6 (Meishan) successive estrous cycles. After a 21-day recovery period, gilts from each line were treated with either a specific GnRH antagonist (SB-75; 10 μg/kg of body weight) or 0.9% saline at 60, 36 and 12 hours prior to slaughter. Blood samples were collected prior to the first treatment and at slaughter before anterior pituitary collection. Serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels were determined by radioimmunoassay and RNA was extracted from anterior pituitary tissue. In all lines, LH was reduced to basal levels by SB-75 treatment, confirming the efficacy of SB-75. In contrast, levels of FSH decreased only in Control gilts following treatment with SB-75. Pituitary levels of GnRH receptor and gonadotropin subunit gene expression were measured by quantitative PCR. Levels of gene expression for the GnRH receptor and gonadotropin subunits decreased following treatment with the GnRH antagonist in pituitaries of gilts from the Index and Control lines; however, these values remained unchanged in pituitaries from Meishan gilts. Identification of unique genetic changes in swine strains with increased ovulation rates, such as the Chinese Meishan and the UNL Index selection line, may allow for a better understanding of prolificacy. This critical information may also be used to enhance litter size in other lines of pigs and improve efficiency of pig production.

Background and Introduction

Prolificacy is an important economic measure of productivity in the pork industry. However, many generations of selection are required to increase the number of live born piglets per litter within an applied breeding program. Thus, it is important to identify the genes and underlying biological mechanisms contributing to increased litter size. While many factors can influence prolificacy, a primary component of litter size is ovulation rate, or the number of oocytes (eggs) available to be fertilized after insemination. From a research perspective, ovulation rate can be measured via visualization of ovaries by surgical procedures (i.e., laparotomy or laparoscopy) or ultrasound. In addition, this trait can be improved in a number of different ways, including nutrition (flushing), hormonal treatment (superovulation), and genetic selection. Despite its importance as a primary component of litter size, however, there is very little known about the genes influencing increased ovulation rate.

Ovulation rate is influenced by circulating levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH), also known as the gonadotropins. The production of these hormones is controlled by the reproductive axis (Figure 1), consisting of the hypothalamus, anterior pituitary gland, and gonads (ovaries or testes). Specifically, gonadotropin-releasing hormone (GnRH), released from the hypothalamus, binds to its receptor on gonadotrope cells of the anterior pituitary gland. Upon binding to its receptor, GnRH stimulates the expression of the GnRH receptor gene itself, as well as the subunit genes that lead to the production of FSH (common alpha- and FSHbeta-subunits) and LH (common alpha- and LHbeta-subunits). The secreted gonadotropins then act on the ovaries to recruit follicles (FSH) or induce ovulation (LH). Steroids, produced by the ovaries, such as estrogen and progesterone provide feedback at the level of both the hypothalamus and anterior pituitary gland to regulate subsequent production of GnRH and gonadotropins, respectively. Therefore, reproductive function is highly dependent on the interaction of GnRH and its receptor.

Sensitivity, or number of GnRH receptors, present on gonadotrope cells of the anterior pituitary gland, may stimulate higher levels of gonadotropin production in lines of swine with increased ovulation rates. In the pig, the sequences for the genes encoding the subunits comprising the gonadotropins, FSH and LH

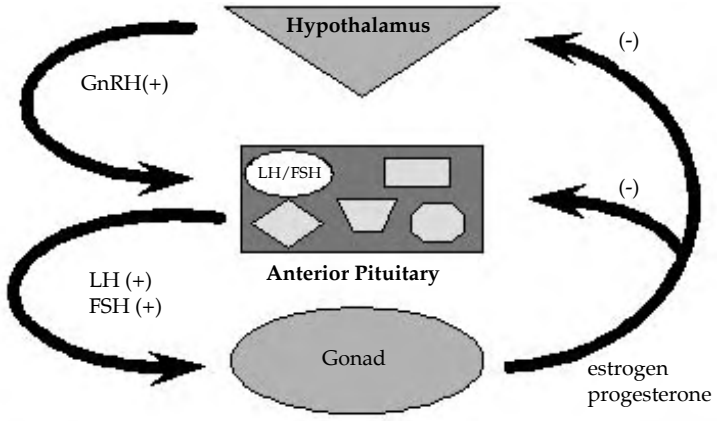

Figure 1. The reproductive axis.

(alpha-, FSHbeta- and LHbeta-subunits), have recently been reported. In addition, the sequence for the porcine GnRH receptor gene was identified by researchers at the University of Guelph. Upon isolation of the gene, investigators at the USDA Meat Animal Research Center have determined that it is located in a similar region as a chromosomal marker for ovulation rate. Therefore, the GnRH receptor gene represents both a physiological and positional candidate for genes influencing ovulation rate in swine. Isolation of these sequences allows for quantification of GnRH receptor and gonadotropin subunit gene expression levels, so comparisons can be made between lines of pigs with ovulation rate differences.

To examine the role of these genes in determining ovulation rate between swine lines, we used swine lines with divergent ovulation rates. Females of the Chinese Meishan breed have a higher ovulation rate than occidental breeds, resulting in four to five more piglets per litter. Thus, Meishan pigs may harbor genetic differences with the potential to enhance reproductive performance of white crossbred pigs. Consistent with the Meishan model, researchers at UNL have developed a line of white crossbred pigs that were selected 11 generations for an index of ovulation rate and embryonic survival, followed by nine generations of selection for increased litter size. At generation 10, females from the UNL Index line ovulated 7.4 more eggs and at generation 19, produced 2.53 more live born piglets per litter than unselected, control animals.

Materials and Methods

Animals and Treatments

All animal procedures conducted in this experimentation were approved by the UNL Institutional Animal Care and Use Committee. White crossbred Index and Control line gilts (Generation 23) were obtained from the University of Nebraska Swine Unit. Gilts of the Meishan breed were obtained from the United States Department of Agriculture, Roman L. Hruska U.S. Meat Animal Research Center in Clay Center, Neb. Gilts were housed in pens with a minimum of 8 square feet of floor space and received 4 pounds of feed per day with water available *ad libitum*. Estrous detection was initiated at 155 days of age for Index and Control gilts and at 95 days of age for Meishan gilts. Upon completion of the third (Index and Control) or sixth (Meishan) estrus, 10 gilts from the Index and Control lines and 12 Meishan gilts were ovariectomized during the luteal phase (day 5 to 15 of the estrous cycle) to remove any confounding effects of steroid hormones (i.e., estrogen) on expression patterns of the genes of interest. During the third estrous cycle, Meishan gilts have similar ovulation rates to that of gilts from occidental breeds. Thus, Meishan females were ovariectomized during the sixth estrous cycle to assure that they would have increased ovulation rates than gilts from the Control line. Eighteen days after ovariectomy, gilts from each line were randomly assigned to treatment groups and received an injection of either the specific GnRH antagonist, SB-75 (10 µg biologically active compound per kg of body weight; UNL Protein Core Facility), or vehicle (0.9% saline) at 60, 36 and 12 hours prior to slaughter. The GnRH antagonist was used to block the effects of GnRH, which is dramatically increased in gilts following ovariectomy. Therefore, GnRH levels were expected to be elevated in ovariectomized gilts treated with vehicle and significantly reduced in ovariectomized gilts treated with the GnRH antagonist, SB-75.

Data Collection

Blood samples were collected prior to ovariectomy and the first treatment, as well as at slaughter. Following slaughter, the anterior pituitary gland was removed. Serum concentrations of FSH and LH were determined using a radioimmunoassay validated in our laboratory. Levels of FSH and LH were determined using a known standard curve of the respective hormone, run simultaneously with the unknown samples. Total RNA, which was extracted from the anterior pituitary tissue, was converted to cDNA and used in quantitative real-time polymerase chain reaction assays to measure expression of the GnRH receptor, glycoprotein alpha-subunit, FSH-beta-subunit and LHbeta-subunit

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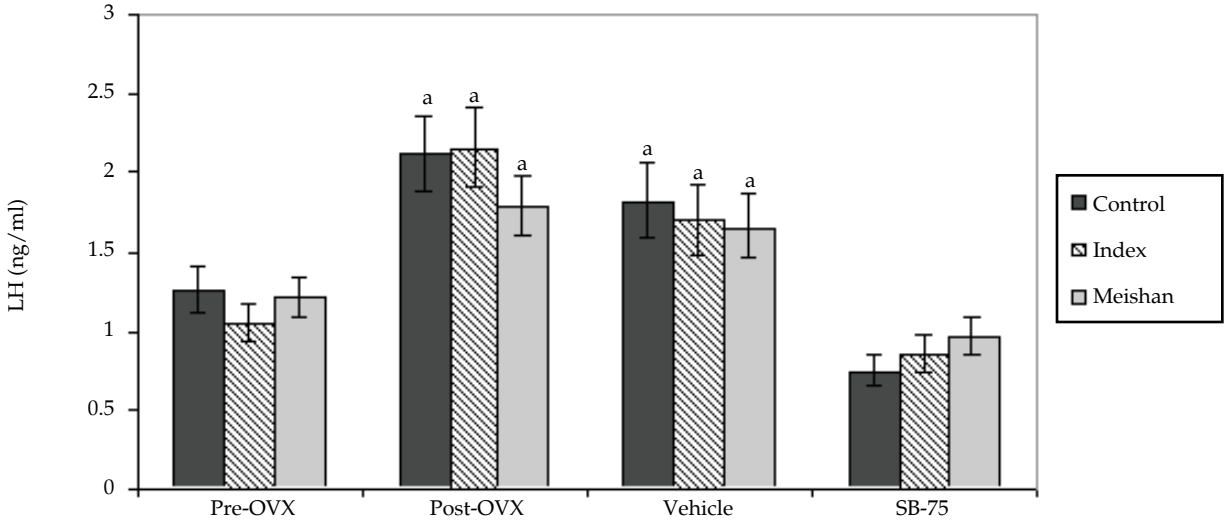

Figure 2. Serum LH levels prior to (Pre-OVX) and after (Post-OVX) ovariectomy and following treatment with the GnRH antagonist, SB-75, or vehicle in Control, Index and Meishan gilts. Each bar represents the least-squares mean ± SEM of 5-6 gilts. Bars with superscripts are different than Pre-OVX groups ($P < 0.05$) and different superscripts indicate differences between lines ($P < 0.05$).

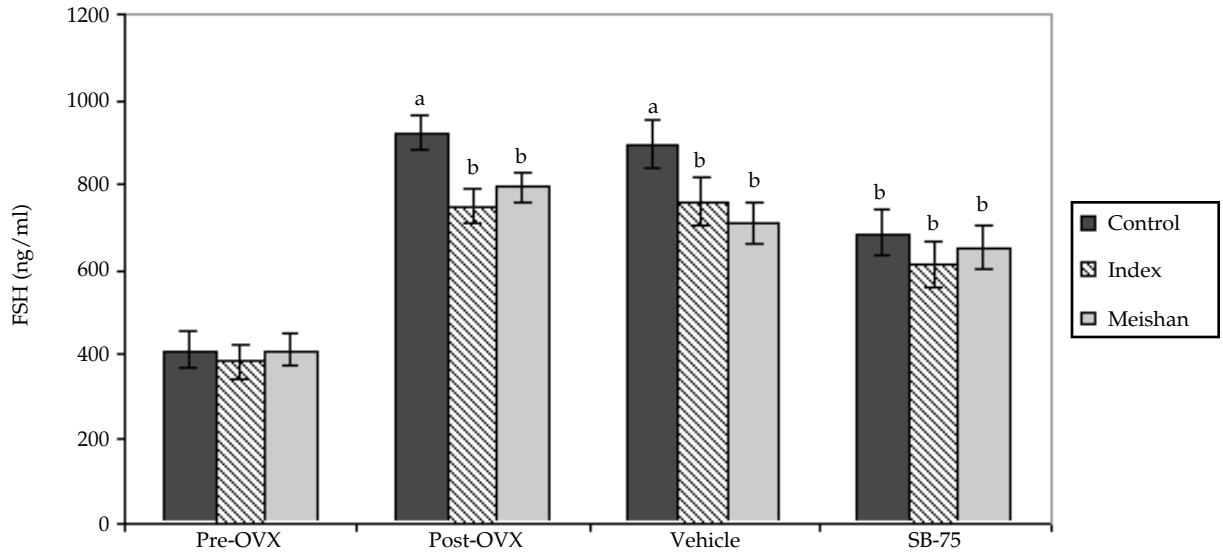

Figure 3. Serum FSH levels prior to (Pre-OVX) and after (Post-OVX) ovariectomy and following treatment with the GnRH antagonist, SB-75, or vehicle in Control, Index, and Meishan gilts. Each bar represents the least-squares mean ± SEM of 5-6 gilts. Bars with superscripts are different than Pre-OVX groups ($P < 0.05$) and different superscripts indicate differences between lines ($P < 0.05$).

genes. The 18s ribosomal RNA kit (Applied Biosystems) was used as an endogenous control gene to normalize each assay.

Statistical Analysis

Statistical evaluation was conducted using the General Linear Models procedure of the SAS. The LH means were logarithmically transformed due to non-normality, analyzed for significance and back-transformed to the original scale. Least-squares means for LH and FSH were compared using least significant differences. Least squares means for expression levels of GnRHR and glycoprotein alpha-subunit, FSHbeta-subunit and LHbeta-subunit genes were logarithmically transformed due to non-normality, analyzed for significance, back-transformed to the original scale and compared using least significant differences. Means for normalized gene expression data are expressed as a ratio of the gene of interest relative to 18s rRNA.

Results and Discussion

Levels of LH (Figure 2) and FSH (Figure 3) were similar in females from all three lines prior to ovariectomy. Following

Table 1. Change in GnRH receptor and gonadotropin subunit gene expression levels following treatment with a GnRH antagonist in lines of swine with differing ovulation rates.

	Genetic Line[a]		
Gene[b]	Control	Index	Meishan
GnRH receptor	Decrease	Decrease	No Change
Common alpha-subunit	Decrease	Decrease	No Change
FSHbeta-subunit	Decrease	Decrease	No Change
LHbeta-subunit	Decrease	Decrease	No Change

[a]Gilts were ovariectomized after the 3rd (Control and Index) or 6th (Meishan) estrus. Following a two-week recovery period, females were treated with the GnRH antagonist, SB-75, or vehicle (0.9% saline) at 60, 36 and 12 hours prior to slaughter.
[b]Changes in gene expression are significant ($P < 0.05$).

ovariectomy, levels of LH were similar among swine lines; however, FSH levels were higher in Control compared to Index and Meishan gilts ($P < 0.05$). Treatment of gilts with the GnRH antagonist, SB-75, decreased LH levels in all lines but levels of FSH decreased significantly only in the Control line compared to animals receiving the vehicle treatment ($P < 0.05$).

Expression of the GnRH receptor gene was greatest in pituitaries of Meishan, intermediate in Index and lowest in Control line gilts treated with vehicle ($P < 0.05$). Treatment with SB-75 reduced expression of the GnRH receptor gene in the Index and Control lines ($P < 0.05$), but not in Meishan gilts (Table 1). Following vehicle treatment, expression of the common glycoprotein alpha-subunit gene was less in pituitaries from Meishan gilts than in Control and Index gilts ($P < 0.05$). Treatment with SB-75 reduced expression of the alpha-subunit gene in pituitaries from females of the Control and Index lines ($P < 0.0001$) but, similar to the GnRH receptor gene, levels of alpha-subunit mRNA in anterior pituitaries of Meishan gilts were unchanged (Table 1). As was observed with the alpha-subunit, expression of the FSHbeta-subunit gene after vehicle treatment was lower in pituitaries of females from the Meishan compared to Control and Index lines. There was a decrease in expression of the FSHbeta-subunit gene in anterior pituitaries of both Control and Index gilts receiving treatment with SB-75 ($P < 0.0001$); however, no change occurred in Meishan gilts (Table 1). After receiving the vehicle treatment, anterior pituitary expression of the LHbeta-subunit gene was lower in Meishan than Control gilts, with Index gilts being intermediate. A decrease in expression of the LHbeta-subunit gene was observed in pituitaries from Control and Index gilts treated with SB-75 ($P < 0.0001$). In contrast, expression of the LHbeta-subunit gene in pituitaries of Meishan females did not decline (Table 1).

Expression of the GnRH receptor, common glycoprotein alpha-subunit, and specific FSHbeta- and LHbeta-subunit genes was reduced in pituitaries of gilts from the Index and Control lines, but not females from the Meishan line following SB-75 treatment. This suggests that differential mechanisms may be involved in gene regulation and production of gonadotropins between Meishan and white crossbred lines of swine. Also, post-treatment expression of the GnRH receptor and LHbeta-subunit genes was significantly greater in anterior pituitaries from females of the Meishan line compared to both Control and Index lines, suggesting that basal expression of these genes is elevated in Meishan gilts. Identification of a trait(s) that could be easily screened and correlated with ovulation rate in young females would be of great interest for selection purposes. Further research needs to be conducted to reveal the mechanisms controlling the observed differences in expression of the GnRH receptor and gonadotropin subunit genes between Meishan and white crossbred lines of swine.

Conclusion

Ovulation rate is very important to swine production, as it is a primary determinant of litter size. Even a modest increase in average litter size of 0.2 pigs per litter on a 10,000 sow operation could net a producer nearly $99,000 in additional profit, depending on pork prices. If differences in pituitary gene expression between Meishan, Index, and Control lines are determined, a region of a particular gene may be isolated to provide a genetic test for ovulation rate. Ultimately, the unique gene sequences from individuals with increased ovulation rates could be incorporated into transgenic swine. This would allow the opportunity to increase ovulation rate in any breed or line of pigs, while maintaining the beneficial characteristics of that breed or line. These animals would be very valuable to pork production worldwide.

[1]Benjamin E. Bass is a graduate student, Rebecca A. Cederberg a research technologist, Ginger A. Mills an agricultural research technician, and Brett R. White an assistant professor in the Animal Science Department at the University of Nebraska–Lincoln.

Effect of Diet and Sire Line on Grow-Finish Performance

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Tom Rathje
John Sonderman[1]

Summary

Two experiments were conducted to determine the influence of sire line and dietary energy levels on grow-finish pig performance. In each experiment, dietary treatments were corn-soybean meal based diets with no added fat and corn-soybean meal based diets with fat added and soybean meal adjusted to maintain a similar lysine: calorie ratio. Fat additions to the added fat diets ranged from 3.75% for the 40 to 70 pound body weight period to 1.5% for pigs over 220 pounds body weight. Within each of five phases during the growing-finishing period, feed budgets were used to maintain a similar total caloric intake between experimental diets. In both experiments, pigs were progeny of Danbred NA 230 females. In Exp. 1, the sire lines compared were Danbred NA 771 versus Danbred NA 671. In Exp. 2, the sire lines compared were Danbred NA 771 versus Danbred NA 600. There were no interactions between sire line and dietary treatment in either experiment. There was no effect of dietary treatment on daily gain. In Exp. 1, feed conversion was improved 6.8% and in Exp. 2, feed conversion was improved 3.7% for the fat added diets versus the control treatment. The lack of daily gain response, when combined with the lack of a genetic interaction, suggests that for these genetic lines daily gain is not a consideration in the decision regarding the use of fat in grow-finish diets.

Introduction

There are numerous reports in the literature detailing the response of barrows and gilts to dietary fat additions during the growing-finishing phase of production. In general, there is almost always an improvement in feed conversion efficiency from the addition of fat to corn-soy based diets. However, an improvement in daily gain is less consistent, especially if the lysine:calorie ratio is similar for the fat added and no fat added diets. Unclear from the literature is whether there is a genetic component to this response. Genetic differences related to feed intake exist; therefore, dietary energy levels necessary to maximize daily gain may differ according to genotype. The following experiment was designed to examine the possible interaction between sire line and dietary fat additions to grow-finish diets.

Materials and Methods

Two experiments were conducted using progeny of the Danbred NA (Columbus, Neb.) 230 female. The experimental diet treatments during the grow-finish phase were:

1. Corn-soybean meal based diets with no added fat (No).
2. Corn soybean meal based diets with added fat (Added).

In Exp. 1, the Danbred NA sire lines compared were 771 versus 671. In Exp. 2, the sire lines compared were 771 versus 600. There were two farrowings of the sire lines within each experiment. Treatments were arranged as a 2 x 2 factorial.

Sire line matings were made at a commercial production unit approximately 200 miles from the research site. The commercial unit was negative for PRRSV. No information is available as to parity distribution of the females used for these matings although an attempt was made to balance sire line matings across parity.

On the day prior to weaning, a representative from Danbred NA identified pigs within litters for use in the experiment. Pigs selected were the heaviest pigs in at least 10 litters and were balanced by sex.

On the day of weaning, 140 pigs (70 from each sire line mating) were transported to the University of Nebraska's Haskell Ag Lab near Concord. At arrival, pigs were ear tagged, weighed, and assigned to nursery pen on the basis of sire line, sex and arrival weight such that within sire line, pens were balanced for sex and similar for arrival weight and coefficient of variation (CV) of arrival weight.

Pigs were housed in 4 x 8 ft nursery pens with woven wire flooring in two nursery rooms. There were five pens per nursery room and the connecting doors between rooms were open. Each pen contained one Drik-o-Mat bowl drinker and one, two-hole Farmweld wean-to-finish feeder. Sire line pens were randomized. There were 14 pigs per pen (2.28 ft^2/pig).

Pigs were fed according to the nursery feed budget detailed in Table 1. Pigs were moved from the nursery on day 34 for both replications in Exp. 1 and on day 35 (replicate 1) and 36 (replicate 2) post weaning in Exp. 2.

Upon removal from the nursery, pigs were moved to a partially slatted grow-finish facility. Facilities were naturally ventilated in the first experiment and mechanically ventilated in the second experiment. Each facility contained 12 6 ft x 15 ft pens. There were 11 or 12 pigs per pen (7.5-8.2 ft^2/pig). There was one nipple drinker and

Table 1. Experimental diets.

	Nursery diets				Grow-finish diets									
Ingredient	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Corn		920	1105	1195	1340.2	1210.3	1430	1304.6	1547.5	1429.1	1644.2	1576.6	1688.3	1644.4
46.5% CP SBM		410	525	645	609.8	665.5	522.6	573.1	407.3	451.5	315.1	337.9	271.6	285.5
Fat[a]		20	20	60	0	75	0	75	0	75	0	45	0	30
Limestone					17.4	16.5	16.7	16.6	16.5	15.8	16.2	16	15.9	16
Dical					15.7	15.5	14.1	13.9	12.7	12.6	10	10	11.7	11.6
Salt					8	8	8	8	8	8	7	7	6	6
Akey 2000[b]	2000													
Akey 650 [b]		650												
Akey 300[b]			350											
Akey 100[b]				100										
Akey 4S Premix[b]					4	4	4	4	4	4	4	4	3.5	3.5
L-lysine					3.5	3.5	3.5	3.5	3.5	3.5	3	3	2.5	2.5
Methionine					0.9	1.2	0.6	0.8	0	0	0	0	0	0
Natuphos 1200G[c]					0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000
Pig wt range, lb					40-70	40-70	70-120	70-120	120-170	120-170	170-220	170-220	220-mkt	220-mkt
Feed budget, lb/pig	1.79	7.14	10.7	20.8	54	51	110	104	135	128	163	158		
Calculated composition														
ME, Kcal/lb		1452	1449	1490	1510	1595	1513	1597	1516	1600	1520	1571	1521	1555
Lysine,%	1.60	1.44	1.37	1.31	1.20	1.26	1.08	1.14	0.93	0.98	0.79	0.81	0.71	0.73
g Lysine/Mcal					3.61	3.59	3.24	3.24	2.79	2.78	2.36	2.34	2.12	2.13

[a]HiEnergy Feed, Des Moines, Iowa.
[b]Akey Inc., Lewisburg, Ohio.
[c]BASF Inc., Florham Park, N.J.

one, two-hole Staco wean-to-finish feeder per pen. Sprinklers were used for summer heat relief with on-off timed sprinkling beginning at 80°F. Following the move to grow-finish, pigs were vaccinated for erysipelas and M hyo. All pigs that died were examined for cause of death by a veterinarian and pen size was not adjusted.

Within sire line, pigs were randomly assigned to grow-finish pens on the basis of weight and sex such that all pens had similar sex ratios and the initial weight and CV for initial weight in all pens was similar.

The experimental diets were formulated to have a constant lysine:calorie ratio within phase. A feed budget was prepared for each diet (Table 1). Feed was budgeted within each phase so as to standardize caloric intake between the no and added fat treatments. The feed budget for the 40-70 lb period was adjusted based on pig weight at the time of relocation from the nursery to the grow-finish facility based on a 1.41 feed:gain for the added fat treatment and 1.51 for the no fat treatment. Prior to relocation, all pigs remained on diet 4 (Table 1).

Because of the difference in arrival weight, pig weight at arrival was used as a covariate in the analysis of nursery performance. Pig weight at the move to the grow-finish facility was used as a covariate in the grow-finish analysis. The pen of pigs was the experimental unit. Within experiment, the model included replicate, sire, diet, and all two- and three-way interactions for grow-finish performance. For nursery performance, the model included replicate, sire, and all two-way interactions.

Results and Discussion

The significance (P = 0.007) in final weight for the 671 vs 771 sired pigs in the first experiment (Table 2) is due in part to the 1.3 lb heavier arrival weight of the 671 sired pigs. There were no interactions between sire lines and diets during the grow-finish phase so the main effects of sire line and diet are presented in Table 3. There was no difference (P > 0.1) in daily gain, daily feed, or feed conversion between the sire lines during the grow-finish phase of the experiment.

As expected, pigs fed diets with added fat during the grow-finish phase had a lower daily feed disappearance (P = 0.003) and improved feed conversion (P < 0.001) compared to pigs fed diets with no added fat during the grow-finish phase of production in Exp. 1. There was no difference in daily gain between the low and high energy diets.

In Exp. 2, there was no effect (P > 0.1) of sire line on nursery performance (Table 4). Similar to Exp. 1, there was no interaction between sire line and diets during the grow-finish phase so the main effects of sire line and diet are presented in Table 5. There was no effect (P > 0.1) of sire line on grow-finish performance.

As in Exp. 1, pigs fed diets containing added fat had a reduction in daily feed (P = 0.001) and an improvement in feed conversion

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(P = 0.007) compared to pigs fed diets with no added fat during the grow-finish phase. There was no difference in daily gain between the low and high energy diets.

The magnitude of the response to added fat varied between experiments. Daily feed was reduced 5.0% for the fat added diets in Exp. 1 and 3.9% in Exp. 2. Feed conversion was improved 6.8% in Exp. 1 while it was only improved 3.7% in Exp. 2 for the fat added diets.

The difference in feed conversion efficiency between the experiments is somewhat surprising. Both replicates of Exp. 1 and the first replicate of Exp. 2 were conducted in winter and spring seasons. Only during the final weeks of replicate 2 of Exp. 2 were the pigs exposed to temperatures above 90°F for extended periods of time. Generally the response to fat additions in diets is greatest when pigs are heat stressed versus grown in thermal-neutral conditions.

Conclusions

While the magnitude of the response to dietary fat additions varied between experiments, the overall improvement in feed conversion efficiency for the pigs fed the fat added diets is in agreement with published results. In both experiments, dietary energy levels higher than typical corn-soybean meal based diets did not result in an improvement in daily gain. The lack of daily gain response, when combined with the lack of a genetic interaction, suggests that for these genetic lines daily gain is not a consideration in the decision regarding the use of fat in grow-finish diets.

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Table 2. Effect of sire line on nursery performance in Exp. 1 – LS means are reported using arrival weight as a covariate, Exp. 1.

	Sire[a]			P value	
Item	671	771	SE	Sire	Sire x trial
No. pens	10	10			
Pig wt, lb					
Arrival	15.6	14.3			
32 day	45.7	42.6	0.5	0.007	0.763
Daily gain, lb	0.963	0.861	0.016	0.006	0.662
Daily feed, lb	1.316	1.288	0.039	0.710	0.583
Feed:gain	1.362	1.493	0.034	0.068	0.299

[a]Danbred NA, Columbus, Neb.

Table 3. Effect of sire line and diet on grow-finish performance in Exp. 1 – LS Means are reported using day 0 weight as a covariate.

	Sire line[a]		Diet[b]			P values		
Item	671	771	Fat	No	SEM	Sire	Diet	Sire x Diet
No. pens	12	12	12	12				
Pig wt, lb								
Day 0	44.9	43.3	44.1	44.1	0.2			
Final[c]	284.5	279.6	284.3	279.7	2.5	0.438	0.212	0.650
Daily gain, lb	1.923	1.885	1.923	1.885	0.020	0.460	0.202	0.638
Daily feed, lb	5.163	5.029	4.965	5.227	0.053	0.327	0.003	0.680
Feed:gain	2.684	2.674	2.585	2.773	0.022	0.863	< 0.001	0.941

[a]Danbred NA, Columbus, Neb.
[b]Fat = added fat per Table 1; No = no added fat.
[c]Day 125 in both trials.

Table 4. Effect of sire line on nursery performance in Exp. 2 - LS means are reported using arrival weights as a covariate.

	Sire Line[a]			P value	
Item	600	771	SE	Sire	Sire x trial
No. pens	10	10			
Pig wt, lb					
Arrival	13.4	14.3	0.04		
Final[b]	47.4	47.3	1.1	0.951	0.761
Daily gain, lb	0.944	0.940	0.030	0.942	0.759
Daily feed, lb	1.357	1.316	0.038	0.583	0.494
Feed:gain	1.436	1.400	0.38	0.583	0.494

[a]Danbred NA, Columbus, Neb.
[b]35 d rep 1; 36 d rep 2.

Table 5. Effect of sire line and diet on grow-finish performance in Exp. 2 – LS Means are reported using day 0 weight as a covariate.

	Sire line[a]		Diet[b]			P value		
Item	600	771	Fat	No	SEM	Sire	Diet	Sire x Diet
No. pens	12	12	12	12				
Pig wt., lb								
Day 0	46.7	47.8	47.4	47.1	0.3			
Final[c]	271.1	269.8	270.4	270.6	2.2	0.737	0.963	0.456
Daily gain, lb	2.045	2.033	2.038	2.040	0.020	0.729	0.951	0.459
Daily feed, lb	5.682	5.634	5.550	5.766	0.041	0.490	0.001	0.814
Feed:gain	2.778	2.774	2.724	2.828	0.025	0.926	0.007	0.255

[a]Danbred NA, Columbus, Neb.
[b]Fat = added fat per Table 1; No = no added fat.
[c]Day 109 and day 110 in trials 1 and 2, respectively.

Effects of Feeding Increased Levels of Vitamin B_{12} to Weanling Pigs

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Summary and Implications

Increasing concentrations of vitamin B_{12} were fed to 144 weanling pigs (weaned 13-14 days) in two, five-week trials. Pigs were fed one of six diets: NC, negative control, basal diet without supplemented vitamin B_{12}; or the basal diet with the inclusion of 100% (1X, 7.94 µg/lb), 200% (2X, 15.87 µg/lb), 400% (4X, 31.75 µg/lb), 800% (8X, 63.49 µg/lb), or 1,600% (16X, 126.98 µg/lb) of NRC requirements for the 11- to 22-lb pig. Each trial was divided into two phases: phase 1, day 0 - day 14 and phase 2, day 14 - day 35. Throughout phase 1, there were no differences among treatments, although ADG (average daily gain) and ADFI (average daily feed intake) increased linearly (P< 0.1). During phase 2, the inclusion of B_{12} resulted in a linear increase (P< 0.05) in ADG with pigs receiving the 16X treatment (126.98 µg/lb) having the greatest gains (ADG = 1.24 lb) in contrast with pigs receiving the control diet (ADG = 1.08 lb). Average daily feed intake increased linearly (P< 0.05) with pigs receiving the control diet consuming less (P< 0.1) than the 2X, 4X, 8X, and 16X treatments during phase 2. Overall (phase 1 and phase 2), ADG increased (P< 0.01) as much as 0.13 lb (16X treatment, 126.98 µg/lb) over the negative control with the inclusion of vitamin B_{12}. Increased concentrations of B_{12} resulted in a linear increase (P< 0.05) in ADG and ADFI overall. This study suggests that feeding levels of vitamin B_{12} above the NRC recommendation may improve weight gain and feed intakes of weanling pigs.

Introduction

Vitamin B_{12}, also known as cobalamin, is a water soluble vitamin that plays a role in two pathways. These pathways are central to energy and amino acid metabolism in animal cells. The pathways in which vitamin B_{12} acts as a coenzyme are: 1) methylmalonyl-CoA synthase, involving the breakdown and utilization of fatty acids and 2) methionine synthase, a reaction in the metabolism of amino acids. Vitamin B_{12} is necessary for the breakdown of odd-chain fatty acids which occur in plant feedstuffs. Vitamin B_{12} plays a major role in amino acid metabolism through DNA methylation and the formation of DNA building blocks, purines and pyrimidines. In previous studies conducted at the University of Nebraska–Lincoln, feeding vitamin B_{12} at concentrations above the 1998 NRC recommendation for the 11- to 22-lb pig resulted in increased average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (ADG/ADFI). This study was conducted to validate previous research and to study the pathways affected by increased supplementation of vitamin B_{12}. Serum homocysteine, vitamin B_{12}, and folate concentrations are being analyzed to determine the role of vitamin B_{12} in the observed growth response.

Materials and Methods

Experimental design

The experiment protocol was reviewed and approved by the Institute for Animal Care and Use Committee of the University of Nebraska–Lincoln. One hundred forty-four pigs were weaned (13 - 14 days), allotted based on initial weaning weight and litter-of-origin, and randomly assigned to one of six dietary treatments. There were four pigs per pen (two gilts/two barrows) and six replications per treatment. Average initial weight was 10.1 lb. The study consisted of two, five-week trials, each divided into phase 1 (day 0 – day 14) and phase 2 (day 14 – day 35).

The six dietary treatments included (Table 1): NC, negative control, basal diet without supplemented vitamin B_{12}; or the basal diet with the inclusion of 100% (1X, 7.94 µg/lb), 200% (2X, 15.87 µg/lb), 400% (4X, 31.75 µg/lb), 800% (8X, 63.49 µg/lb), or 1,600% (16X, 126.98 µg/lb) of NRC requirements for the 11- to 22-lb pig.

Live animal care and measurements

Pigs and feeders were weighed weekly for determination of ADG, ADFI, and ADG/ADFI. Blood was collected each week for analysis of serum (still in progress) vitamin B_{12}, folate, and homocysteine. Mats and heat lamps were placed in pens for phase 1 and removed for the remainder of the trial.

Statistical analysis

Data were analyzed as a completely randomized block design using the MIXED procedure of SAS. The main effect of the statistical model was dietary treatment. Pen was the experimental unit used for analyses. Pairwise comparisons were made to observe differences among treatments for ADG, ADFI, and ADG/ADFI.

(Continued on next page)

Table 1. Composition of phase 1 and phase 2 dietary treatments (as-fed basis)

	Phase 1[1,2]						Phase 2[1,3]					
Ingredients, %	NC	1X	2X	4X	8X	16X	NC	1X	2X	4X	8X	16X
Corn	31.81	31.81	31.81	31.81	31.81	31.81	45.09	45.09	45.09	45.09	45.09	5.09
Soybean meal, 46.5% CP	10.63	10.63	10.63	10.63	10.63	10.63	30.59	30.59	30.59	30.59	30.59	0.59
Soy protein concentrate	6.25	6.25	6.25	6.25	6.25	6.25	0.00	0.00	0.00	0.00	0.00	0.00
Whey, dried	30.00	30.00	30.00	30.00	30.00	30.00	14.99	14.99	14.99	14.99	14.99	4.99
Animal plasma	8.00	8.00	8.00	8.00	8.00	8.00	2.00	2.00	2.00	2.00	2.00	2.00
Blood cells	0.00	0.00	0.00	0.00	0.00	0.00	3.00	3.00	3.00	3.00	3.00	3.00
Lactose	4.00	4.00	4.00	4.00	4.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00
Dicalcium phosphate	1.28	1.28	1.28	1.28	1.28	1.28	1.60	1.60	1.60	1.60	1.60	1.60
Limestone	0.69	0.69	0.69	0.69	0.69	0.69	0.53	0.53	0.53	0.53	0.53	0.53
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00	3.00	3.00	3.00	3.00	3.00	3.00
Mecadox®	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
UNL mineral mix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
UNL vitamin mix[5]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Zinc oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine • HCl	0.15	0.15	0.15	0.15	0.15	0.15	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin $B_{12,}$ µg/lb	0.00	7.94	15.87	31.75	63.49	126.98	0.00	7.94	15.87	31.75	63.49	6.98

[1] N -
quirement (31.75 µg/lb), 8X = 800% of NRC requirement (63.49 µg/lb), 16X = 1,600% of NRC requirement (126.98 µg/lb).
[2]Phase 1 diets formulated to contain: lysine, 1.60%; Ca, 0.91%; P, 0.80%; available P, 0.57%.
[3]Phase 2 diets formulated to contain: lysine, 1.42%; Ca, 0.85%; P, 0.75%; available P, 0.45%.
[4]Supplied per kilogram of diet: Zn (as ZnO), 128 mg; Fe (as $FeSO_4 \bullet H_2O$), 128 mg; Mn (as MnO), 30 mg; Cu (as $CuSO_4 \bullet 5\ H_2O$), 11 mg; I (as $Ca(IO_3) \bullet H_2O$), 0.26 mg; Se (as Na_2SeO_3), 0.3 mg.
[5]UNL vitamin mix excluding vitamin B_{12}. Supplied per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 440 IU; α-tocopheryl acetate, 24 IU; menadione sodium bisulfite, 3.5 mg; riboflavin, 8.8 mg; d-pantothenic acid, 17.6 mg; niacin, 26.4 mg.

Results and Discussion

Figures 1a-c show the growth criteria responses to dietary treatments. There were no treatment effects on ADG (0.50 lb), ADFI (0.73 lb), or ADG/ADFI (1.52 lb/lb) during phase 1, although there were linear effects of B_{12} addition on ADG and ADFI ($P < 0.1$). During phase 2, pigs receiving the negative control, NC, (ADG = 1.08 lb) had lower ($P< 0.05$) ADG than all other treatments with the pigs receiving the 16X treatment having the greatest ADG (1.24 lb). There was a linear response ($P< 0.05$) of ADG to B_{12} addition during phase 2. Pigs receiving the negative control consumed less feed ($P < 0.05$; ADFI = 1.59 lb) than the 2X, 8X, and 16X treatments during phase 2. Pigs receiving the 4X treatment had numerically greater ADFI ($P< 0.10$; ADFI = 1.69 lb) than pigs not receiving vitamin B_{12} supplementation. Pigs receiving the 1X treatment had greater ($P< 0.05$) feed efficiency (ADG/ADFI = 1.58 lb/lb) than pigs receiving the NC (ADG/ADFI = 1.50 lb/lb) during phase 2. Pigs receiving the 2X and 16X diets had numerically greater ($P< 0.1$) ADG/ADFI than the NC. The addition of B_{12} resulted in increased ($P< 0.01$) ADG and increased ($P< 0.10$) ADFI and ADG/ADFI, overall. Pigs receiving the NC had lower ADG, ADFI, and ADG/ADFI than those receiving other treatments.

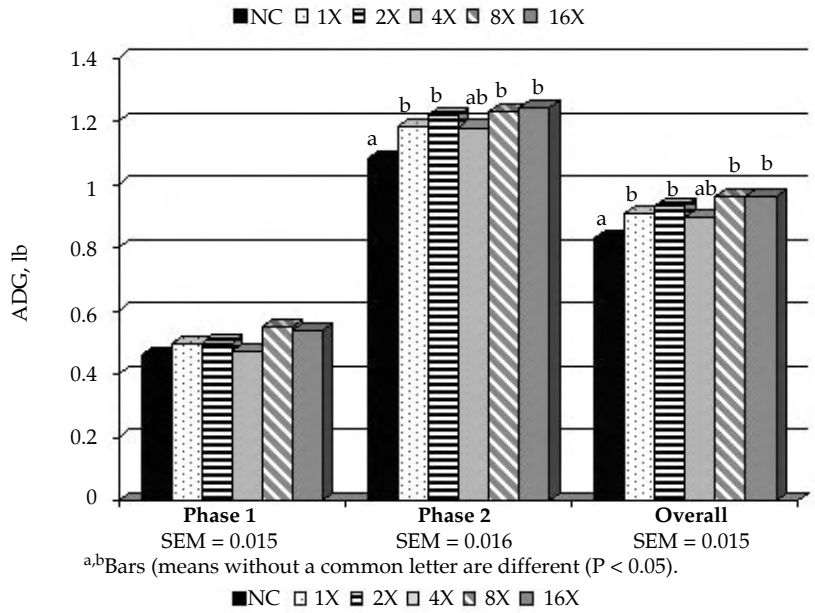
Pigs supplemented with vitamin B_{12} had greater ADG, ADFI, and feed efficiency than those not supplemented with vitamin B_{12} in this study, and in research previously conducted at the University of Nebraska–Lincoln. Similar to other studies, no treatment effects for ADG, ADFI, or ADG/ADFI were observed in phase 1. This was likely due to storage of vitamin B_{12} in pigs. The pigs receiving the 4X treatment did not perform as well as other pigs receiving supplemental vitamin B_{12}. Overall, pigs with the greatest gains (ADG = 0.96 lb) and greatest intakes (ADFI = 1.35 lb) were on the 16X dietary treatment, while the 1X treatment had the greatest ADG/ADFI (1.57 lb/lb).

Conclusion

This study suggests that by feeding weanling pigs vitamin B_{12} above the NRC recommendation for the 11- to 22-lb pig may increase weight gain and feed intakes. The results of this study are similar to those of previous studies from our research group. Subsequently, we plan to measure vitamin B_{12}, folate, and homocysteine in serum and re-evaluate the growth performance data in the context of the serum analyses.

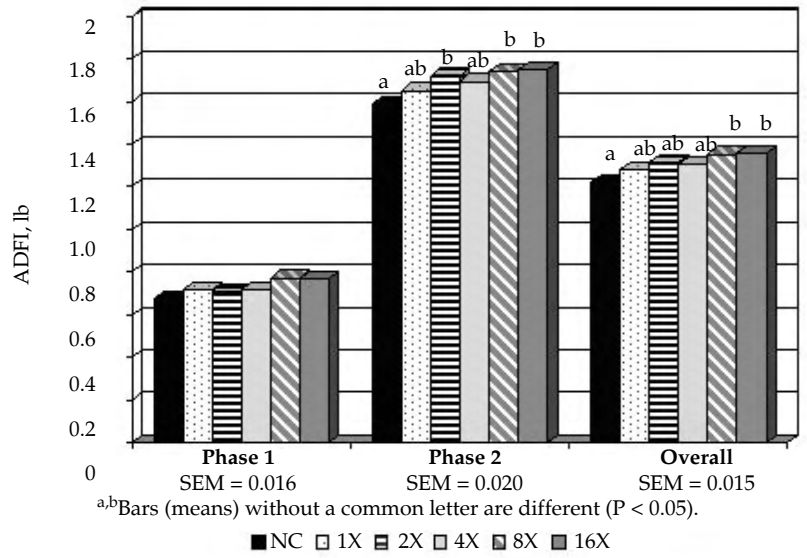
[1]Laura R. Albrecht is a graduate student, Robert L. Fischer is a former graduate student and research technologist, and Philip S. Miller is a professor in the Animal Science Department.

a)



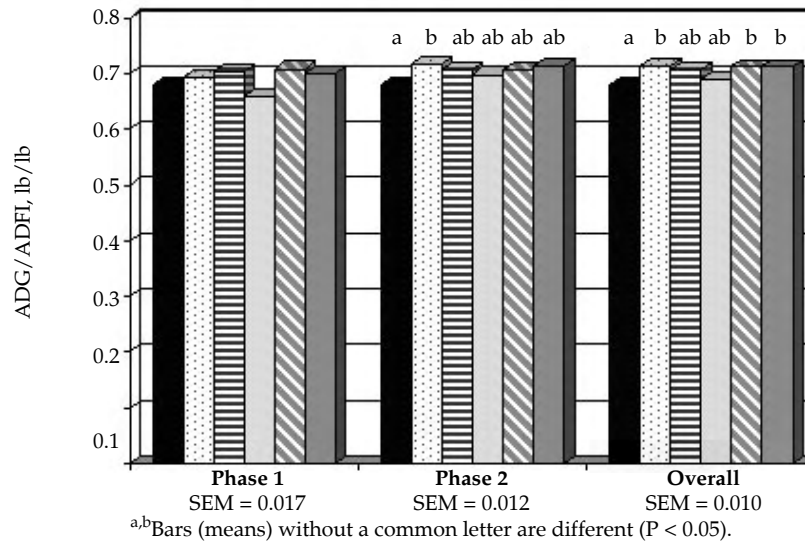
[a,b]Bars (means without a common letter are different (P < 0.05).

b)



[a,b]Bars (means) without a common letter are different (P < 0.05).

c)



[a,b]Bars (means) without a common letter are different (P < 0.05).

Figure 1. Phase 1, phase 2, and overall growth responses of 10- to 45-lb pigs. a) ADG (average daily gain), b) ADFI (average daily feed

(63.49 µg/lb), and 16X = 1,600% (126.98 µg/lb) of NCR requirements for the 11- to 22-lb pig. SEM = standard error of the mean.

Effects of Nutrition During Gilt Development on Lifetime Productivity of Sows of Two Prolific Maternal Lines: Growth and Puberty Characteristics of Rep 1 Gilts

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Summary

This report is an annual update of an ongoing experiment initiated in 2005 to investigate effects of energy restriction during gilt development on reproduction through four parities. Gilts of two genetic lines expected to differ in rate of growth are used and are developed with either ad libitum access to feed or are restricted in energy to 75% of ad libitum amounts from approximately 120 days of age to breeding. Semen of the same sires, an industry maternal line, was used to produce gilts of both lines, but their dams were from two uniquely different populations. Dams of one line were an industry Large White x Landrace (LW x LR) cross and dams of the other line were from a Nebraska line (Line 45) selected 23 generations for increased ovulation rate, uterine capacity, and litter size (L45X). Both lines are expected to be prolific, but L45X females are expected to be extra prolific, being earlier maturing and having larger litters; whereas LW x LR gilts are expected to have greater rates of lean growth. The experiment is being conducted in three replications with 160 gilts per replication. Replication 1 gilts completed the gilt development phase in summer of 2005 and were mated for December 2005 litters. Replication 2 gilts were born in May 2005 and are currently in the gilt development phase. Replication 3 gilts will be born in November 2005. The project will terminate when Replication 3 females wean their fourth parity litters. This report summarizes growth rate, backfat and longissimus muscle deposition, and age at puberty in Replication 1 gilts. Lines differed in growth rate, LW x LR cross gilts grew faster than L45X gilts, but at the same weights, lines had similar backfat and longissimus muscle area. L45X gilts were younger at puberty. Restricting intake during the gilt development period affected both lines similarly, reducing growth rate and backfat deposition, but did not affect longissimus muscle deposition. The objectives of the experiment are being accomplished and will answer the question of whether energy restriction during gilt development, and thus less backfat at breeding, affects lifetime productivity.

Introduction

Annual death losses in many sow herds average 10 to 12%, and losses as high as 18% have been recorded. Death losses and involuntary culling result in annual sow replacement rates of 45 to 55%. Because lower sow culling rates would have important economic benefits for pig producers, the Animal Science Committee of the National Pork Board has identified sow longevity/mortality as an industry priority for 2006.

Many variables contribute to herd-to-herd variation in sow mortality including housing systems, management practices associated with gilt development, sow management practices, and possibly use of different genetic lines. At the University of Nebraska–Lincoln (UNL), we are focusing on two of these components: nutritional regimens during gilt development and prolific lines that differ in rate of lean growth.

Two gilt development practices prevail in the industry. One is to provide gilts ad libitum access to feed for maximum growth rate until 230 to 250 lb; thereafter gilts are limit fed until flushing/breeding at 280 to 300 lb. Another practice is to maintain gilts with ad libitum access to feed right up to breeding. In both cases, it is commonplace to mate gilts at their second or third post-pubertal estrus and mate them again for subsequent litters within five to 10 days of weaning after a 15 to 23-day lactation period.

Optimum gilt development regimens, however, may depend on the prolificacy of the line and on its rate of lean growth. We initiated an experiment to address the effects of different nutritional regimens during gilt development on sow reproduction and longevity. The initial report of the design of the experiment is in the 2005 Nebraska Swine Report. The experiment was designed to determine whether gilt nutritional development strategies affect longevity and lifetime productivity of prolific gilts that differ in rate of lean growth. Sow longevity was defined as production through four parities. The time between when females are mated to produce project gilts until gilts wean their fourth litter is just over two years. The project is being conducted in three replicates at

approximately four-month intervals; therefore, the entire experiment will take approximately three years to complete. Replication 1 gilts completed the development phase during the summer of 2005 and were mated during September of 2005. This report presents the feed intake, growth rate, and puberty data for Replication 1 gilts.

Materials and Methods

Production of Replication 1 gilts

Litters from which Replication 1 gilts were selected were born during the last week of December 2004 and the first week of January 2005. Their dams were from two distinctly different maternal lines (see below) that were inseminated during a two-week period in September of 2004 with semen from boars of an unrelated industry maternal line (L_M). Project gilts were selected randomly when pigs were 56 days of age.

Gilt Population I (LW x LR): Population 1 gilts were the progeny of L_M boars and females of the Large White-Landrace female population that is used routinely in the UNL swine nutrition research program. It is maintained using artificial insemination in a rotation cross between the industry Large White (LW) and Landrace (LR) lines. These females are designated as industry LW x LR cross. A total of 20 litters of this cross were produced; 80 LW x LR gilts, averaging four per litter, were selected for Replication 1.

Gilt Population II (L45X): Population 2 gilts were progeny of the same L_M boars that sired LW X LR gilts and females of the Nebraska line (Line 45) that has been selected 23 generations for increased litter size. This population is designated L45X. Selection over the generations in the Nebraska line included combinations of ovulation rate, uterine capacity, and litter size at birth. During the last six generations, Line 45 also was selected for increased growth rate, decreased backfat, and increased longissimus muscle area. A total of 45 L45X litters were produced; 80 gilts, two from each of 40 litters, were selected for Replication 1.

All litters were sired by a total of nine L_M boars. Thus, the 160 gilts selected for Replication 1 represented nine half-sib families that contained both LW x LR and L45X gilts.

Management of gilts

At birth, pigs from litters the gilts were born in were crossfostered both within and between sows of the two populations to reduce variation in number of pigs nursed by dams. Litters averaged 13.3 days of age at weaning (range 11 to 16 days). At weaning, pigs were placed in a nursery with 30 pigs per pen where they remained until approximately 56 days of age. Standard nursery diets and management were used.

At an average of 56.2 days of age (range of 48 to 61 days), gilts were weighed and placed in pens of 10 head per pen by population, age, and litter in a modified-open-front, curtain-sided building (MOF). All pens were identical with 1/3 slatted and 2/3 solid surface, providing approximately 8.5 sq ft per gilt. Gilts of LW x LR and L45X populations were assigned to alternate pens and littermates were assigned to different pens (e.g., Pens 1 and 3 contained littermates, Pens 2 and 4 contained littermates, etc.) Within each of these pairs of pens within populations, one pen was randomly assigned to Treatment 1 (see below), the other received Treatment 2, resulting in four pens per population x treatment class.

Treatments. Gilts received the same diet and management from when they were placed in the MOF until an average age of 123 days. During that time, they had ad libitum access to a standard corn-soybean meal diet. A three-phase feeding regimen was used. Phase 1 diet contained 1.15% lysine and was fed from 56 days of age to 80 lb, Phase 2 diet contained 1.0% lysine and was fed from 80 lb to mean weight of 130 lb, Phase 3 diet contained .9% lysine and was fed until gilts were 123 days of age when they were placed on experimental dietary regimens.

Treatment 1 was a feeding regimen in which gilts were provided ad libitum access to feed in a self-feeder during the entire period from 123 days of age until they were moved to the breeding barn approximately one week before breeding commenced. The diet was corn-soybean meal-based and formulated to contain 0.70% lysine, 0.70% Ca, and 0.60% P. All other nutrients met or exceeded requirements for developing gilts outlined in the UNL/SDSU Swine Nutrition Guide (2000).

Gilts on Treatment 2 received a daily allotment of feed by weight that was 75% of that consumed by gilts on Treatment 1. The diet was formulated similar to the diet described for Treatment 1 except that it was fortified to contain 0.93% lysine, 1.0% Ca, and 0.8% P. All trace minerals, except Se, and vitamins were also increased to compensate for reduced feed intake. Daily intake of all nutrients except energy was expected to be similar for gilts on both diets. The daily allotment was adjusted at two-week intervals and was based on average daily feed intake of gilts of the same population with ad libitum access to feed.

Beginning at 56 days of age, gilts were weighed at two-week intervals, feed delivered to each pen during that interval was recorded, and beginning and ending feeder weights were recorded. Average daily feed intake (ADFI) for pens of gilts with ad libitum access to feed (T1 and T2 before 123 days of age, T1 after 123 days of age) in each pen during each

(Continued on next page)

two-week period, and the mid-weight (MW) of gilts in that pen (mean beginning weight + mean final weight)/2) were calculated. After each weigh-day, quadratic regression of ADFI on MW was calculated separately for LW x LR and L45X gilts. Beginning at 123 days of age, predicted MW of gilts in each pen on Treatment 2 during the next two-week period was calculated from past growth and used in the regression equation to calculate the expected average feed intake for the pen if ad libitum access to feed was permitted. The average daily allotment for gilts in that pen during the next period was set at 75% of that value. The allotment of feed was placed on the solid flooring daily in two feedings, one-half at approximately 8:00 a.m. and one-half in late afternoon.

Traits. Pigs averaged 56.2 days at the beginning of the trial and 235 days when last weights were recorded. Fourteen weights per pig and 13 pen feed intake values were recorded. When pigs were placed on treatment at 123 days of age, ultrasound scans of backfat (BF) and longissimus muscle area (LMA) at the 10th rib also were recorded. There were nine BF and LMA records per pig.

Beginning when mean age of pigs in each pen was 140 days, heat-checking to determine age at puberty commenced. It was accomplished by moving pigs from each pen to an adjacent building where they were exposed to a boar and observed for the standing response indicative of estrus. The day of first observed estrus was considered to be age at puberty. Heat checking continued until the end of the trial or until all gilts in the pen had been observed in estrus at least twice. Length of estrus, the number of consecutive days they remained in estrus, and the intervals between estrous periods were recorded. Gilts were moved to the breeding facility at approximately 240 days of age.

Analyses: Feed intake, weight, backfat, longissimus muscle area, and puberty data of Replication 1 gilts are reported. Regressions of feed intake on MW over the entire feeding period were compared between LW X LR and L45X. No other feed intake comparisons were made because feed intake for gilts on Treatment 2 was controlled.

Age at puberty was analyzed with a model including population, dietary treatment, their interaction, and the random effect of litter as the error variance. Other variables were analyzed with regression methods. Weight was regressed on age in a model including fixed effects of population, dietary treatment, and their interaction and linear and quadratic regressions on age and interactions of regression coefficients with fixed effects. Litter was included as a random effect and repeated measures on each pig were accounted for. Similar analyses were performed for backfat and longissimus muscle area, except that these variables were regressed on weight.

Table 1. Numbers of gilts starting the trial, numbers removed because they were unthrifty, numbers expressing their pubertal estrus, and mean age at puberty for gilts in each group.

Population	Nutritional regimen	No. at 56 days of age	No. removed 56 to 123 days (unthrifty)	No. removed 123 to 236 days (unthrifty)	No. expressing pubertal estrus	Mean age at puberty
LW X LR	Ad libitum	40	0	0	38	173.2
	Restricted	40	0	0	34	167.5
L45X	Ad libitum	40	3	1	35	161.1
	Restricted	40	2	0	37	167.3
Population						*
Population *treatment						*

LW X LR = Cross of commercial L_M boars with UNL Large White-Landrace females.
L45X = Cross of L_M boars with females of the Nebraska prolific line.
*P < 0.05.

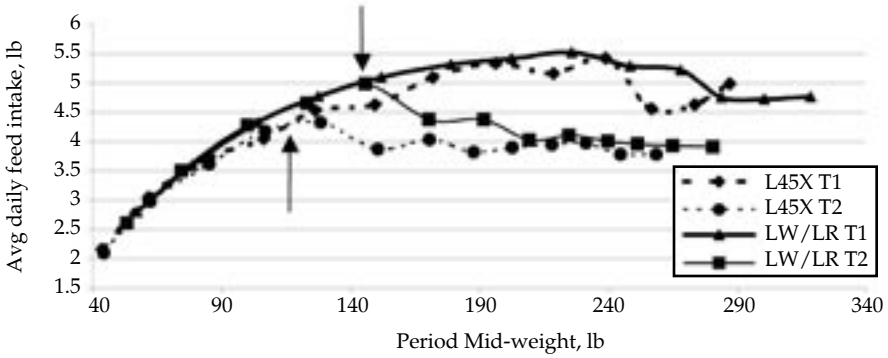

Figure 1. Average daily feed intake plotted against mid-weight per two-week period for LwxLR (solid lines) and L45X (dashed lines) gilts developed with ad libitum intake (T1 = bold lines) or 75% of ad libitum intake (T2 = plain lines) from 120 days of age.

Results

Table 1 contains the numbers of unthrifty gilts that were removed from the trial, the numbers for which a pubertal estrus was recorded, and the mean age at puberty for gilts in each group. Six unthrifty L45X gilts were removed from the trial before breeding age, five of them were removed before

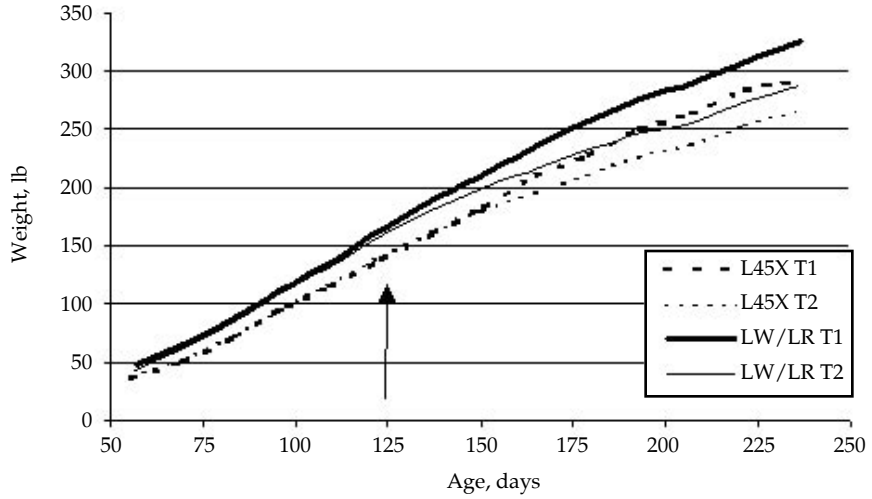

Figure 2. Regressions of weight on age for LW x LR (solid lines) and L45X (dashed lines) gilts developed on ad libitum access to feed (T1 = bold lines) or 75% of ad libitum (T2 = standard lines) from 120 days of age.

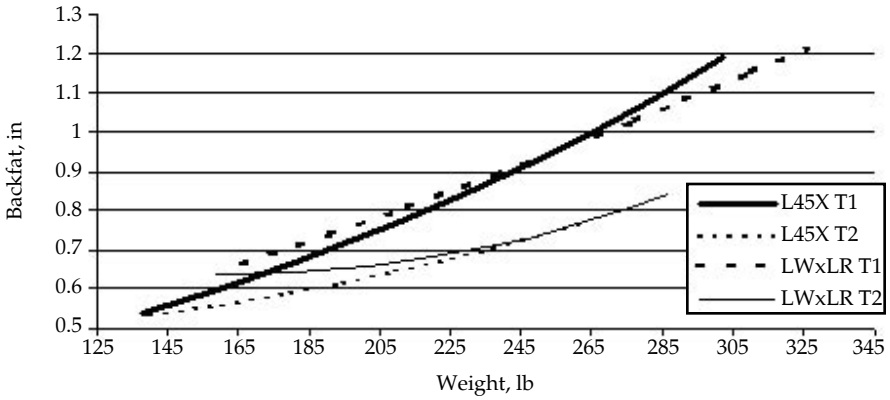

Figure 3. Regressions of backfat on weight for LW x LR (solid lines) and L45X (dashed lines) cross gilts developed with ad libitum intake (T1 = bold lines) or 75% of ad libitum intake (plain lines) from 123 days of age.

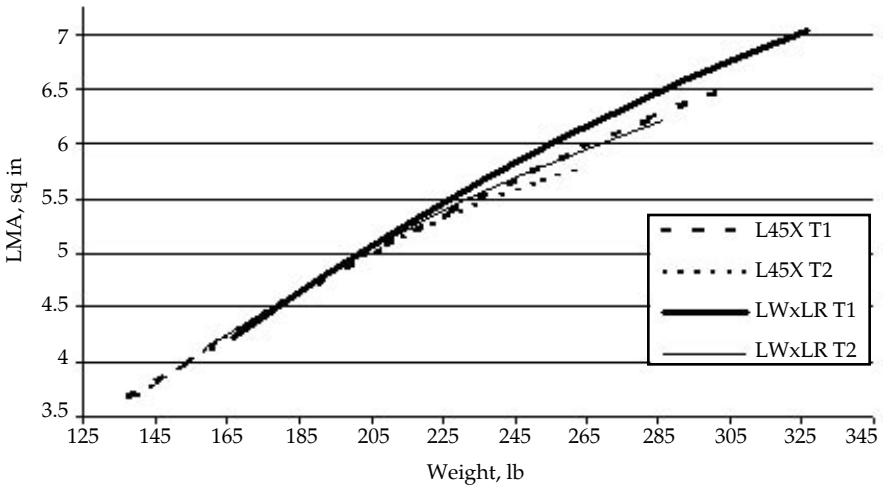

Figure 4. Regressions of longissimus muscle area on weigh for LwxLR (solid lines) and L45X (dashed lines) gilts developed with ad libitum intake (T1 = bold lines) or 75% of ad libitum intake (T2 = plain lines) from 123 days of age.

123 days of age when nutritional treatments began. Of the remaining gilts, 72 of 80 LW X LR gilts and 72 of 74 L45X gilts were observed in estrus. Overall, L45X gilts were 6.1 days younger at puberty than LW x LR gilts ($P < 0.05$); nutritional regimen did not affect age at puberty. However, a population x treatment interaction existed ($P < 0.05$) as LW X LR gilts developed on restricted feed intake were 5.7 days younger at puberty than those developed with ad libitum access to feed, whereas L45X gilts developed on ad libitum access to feed were 6.2 days younger than those on restricted intake.

Figure 1 illustrates average daily feed intake plotted against mid-weight during each 14-day period. Feed intake for LW X LR and L45X gilts with ad libitum access to feed was similar, increasing in a curvilinear fashion from average intake of approximately 2.2 lb per day when gilts weighed 43 lb and increasing to a maximum of approximately 5.6 lb per day when gilts weighed 230 to 240 lb. Hot weather during July and August may have contributed to the decline in intake after 240 lb. Because of more rapid growth (Figure 2), LW X LR gilts were heavier than L45X gilts when feed restriction was imposed (indicated by arrows in Figure 1) and remained heavier during each subsequent period.

The experiment was designed so that predicted feed intake of restricted-fed gilts was 75% of the intake of gilts with ad libitum access to feed. However, they actually consumed somewhat more, averaging 80% for LW X LR gilts and 78% for L45X gilts over the entire period of feed restriction.

The plot of weight against age (Figure 2) illustrates growth rate for gilts in each population by treatment class. Population, nutritional treatment, and interaction all affected growth rate ($P < 0.05$). LW X LR gilts grew faster than L45X

(Continued on next page)

gilts, were heavier at all ages, and the difference increased with age. Dietary treatment suppressed rate of growth so that at breeding age, gilts on restricted intake weighed 88% (LW X LR) and 90% (L45X) as much as their littermates with ad libitum access to feed.

Figures 3 and 4 illustrate the increase in 10th rib backfat thickness and longissimus muscle area relative to body weight for gilts of each class. Backfat per unit of live weight was similar at all weights for LW X LR and L45X gilts, and restricting intake reduced backfat ($P < 0.05$) similarly in gilts of both populations. At 235 days of age, backfat of gilts on the restricted-intake regimen was 70% (LW X LR) and 65% (L45X) of that of their littermates with ad libitum access to feed. Longissimus muscle area relative to body weight, however, was similar for gilts of both populations and was not affected significantly by nutritional regimen.

Discussion

Growth rates and backfat and longissimus muscle development for LW x LR and L45X gilts with ad libitum access to feed are consistent with previous data for these populations. At the same weights, gilts of the two populations have similar backfat and longissimus muscle; but LW x LR gilts grow faster and, therefore, have greater rates of lean growth. The objective in designing the nutritional regimens was to provide a diet with restricted energy but that provided similar daily amounts of lysine, vitamins and minerals so that rate of fat deposition would be decreased with little or no reduction in rate of muscle deposition. Figures 3 and 4 illustrate that this objective was accomplished.

The main project objective is to evaluate the long-term effects of these gilt development regimens on productivity through four parities. Replication 1 gilts were mated in September of 2005. Their breeding performance and their litter productivity will be reported in the 2007 Nebraska Swine Report. Replication 2 gilts were born in May of 2005, and available data on them will be included in that report.

[1]Beth Maricle is an undergraduate animal science student; Matthew W. Anderson is manager of the University of Nebraska–Lincoln Swine Research Farm; Jeffrey Perkins and Donald R. McClure are research technicians at the UNL Swine Farm; Laura R. Albrecht and Roman Moreno are animal science graduate students; Phillip S. Miller and Rodger K. Johnson are professors of the Animal Science Department.

Effect of Low-Protein Non-Amino Acid Supplemented Diet and Ractopamine (Paylean®) on Growth Performance and Serum Urea Concentration of Late-Finishing Pigs

Roman Moreno
Robert L. Fischer
Phillip S. Miller[1]

Summary and Implications

When feeding excessive amounts of protein, the nitrogen eliminated by the pigs in swine facilities has an important impact in the environment. Therefore, it is important to define nutritional strategies that promote a more efficient use of protein. This study was conducted to evaluate the effect of a low-protein non-amino acid supplemented diet and ractopamine (Paylean®) on performance of late-finishing pigs. Thirty-six finishing barrows and gilts with an initial body weight of 153.4 lb were used in a 42-day experiment. Pigs were penned individually and had ad libitum access to feed and water. The pigs were randomly allotted to one of four dietary treatments with different dietary protein (10 or 16 % CP) and ractopamine (0 or 20 ppm) concentrations. Body weight and feed disappearance were measured weekly. Average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (ADG/ADFI) were calculated. Blood samples were collected weekly by venipuncture and serum was collected. Data were analyzed as repeated measures and by orthogonal contrast (to examine differences among means). There were treatment differences for ADG ($P < 0.05$) for the overall experimental period with the highest ADG (2.26 lb/day) corresponding to the pigs receiving 16% CP and 20 ppm ractopamine. There was no ractopamine effect on serum urea nitrogen (SUN) for any weekly period or overall. Average daily feed intake was lower for diets with 16% CP compared to diets with 10%

Table 1. Ingredient and calculated nutrient composition of the experimental diets, as-fed basis.

Item, %	Dietary Protein Concentration, %			
	10 + 0 ppm RAC[a]	10+ 20 ppm RAC	16 + 0 ppm RAC	16 + 20 ppm RAC
Corn	89.1	89	74.02	73.92
Soybean meal, 46.5% CP[b]	5.5	5.5	20.75	20.75
Tallow	3	3	3	3
Dicalcium phosphate	1.05	1.05	0.95	0.95
Limestone	0.7	0.7	0.625	0.625
Salt	0.3	0.3	0.3	0.3
Vitamin mix[c]	0.2	0.2	0.2	0.2
Trace mineral mix[d]	0.15	0.15	0.15	0.15
Paylean®	—	0.1	—	0.1
Calculated composition				
ME, Mcal/lb[e]	1.58	1.57	1.58	1.57
CP, %	10	10	16	16
Total lysine, %	0.39	0.39	0.77	0.77
Calcium, %	0.6	0.6	0.6	0.6
Available phosphorus, %	0.23	0.23	0.23	0.23

[a]RAC = Ractopamine.
[b]CP = Crude protein.
[c]Supplied per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 440 IU; a-tocopheryl acetate, 24 IU; menadione sodium bisulfite, 3.5 mg; riboflavin, 8.8 mg; d-pantothenic acid, 17.6 mg; niacin, 26.4 mg; vitamin B_{12}, 26.4 mg.
[d]Supplied per kilogram of diet: Zn (as ZnO), 128 mg; Fe (as $FeSO_4 \bullet H_2O$),128 mg; Mn (as MnO), 30 mg; Cu (as $CuSO_4 \bullet 5\ H_2O$), 11 mg; I (as $Ca(IO_3)H_2O$), 0.26 mg; Se (as Na_2SeO_3), 0.3 mg.
[e]ME = Metabolizable energy.

CP, (P < 0.05). For diets with 10% CP (vs. 16% CP), ADG/ADFI was lower (P < 0.05). There was an effect of protein on ADG and ADG/ADFI (P < 0.05), but not on SUN or ADFI. The lack of an effect of ractopamine on ADG, ADFI, ADG/ADFI or SUN was possible due to an inadequate protein or amino acid intake. Ractopamine tended to increase growth performance. In summary, the highest CP concentration used in this experiment failed to provide an adequate amino acid supply to allow ractopamine to increase growth performance of late-finishing pigs. It appears that ractopamine requires dietary CP concentrations greater than 16% to improve growth performance in late-finishing pigs from the University of Nebraska–Lincoln herd.

Introduction

The amount of protein retained by finishing pigs is a function of the quality and amount of protein consumed, as well as the body weight and age of the pigs. Feeding pigs with the adequate amount of protein and a correct balance among the essential amino acids help avoid feeding excess protein that would increase the need to eliminate nitrogen (N). Excess N eliminated by pigs in swine facilities has an important impact on the environment because N can contaminate soil and underground water supplies.

Researchers have investigated the effects of low-protein diets on finishing pig performance as a means to improve the efficiency of protein use and to reduce N excretion. Recent investigations have shown that a 1% reduction in dietary protein concentration for finishing pigs resulted in a 10% reduction in the N excretion. Researchers have also shown that growth performance is maintained when pigs are fed diets containing 4% less protein (amino acid-supplemented) compared to pigs receiving a complete corn-soybean meal diet.

The goal of the present investigation was to determine if feeding late-finishing pigs standard or low-protein non-amino acid-supplemented diets with or without ractopamine (Paylean®) results in similar growth performance. This was a preliminary study designed to establish a response range for dietary crude protein (CP) and ractopamine additions for pigs from the UNL herd.

Procedures

Animals and treatments

Thirty-six crossbred [Danbred × (Danbred × Nebraska white line)] late-finishing barrows and gilts were used in a 42-day experiment. The average initial weight was 153.4 lb and the final weight was 234.0 lb. Pigs were penned individually in fully-slotted pens with ad libitum access to feed and water. The room was maintained at 72°F. All management and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Nebraska–Lincoln.

Experimental diets

Treatments were arranged as a 2 x 2 factorial. The pigs were randomly assigned to one of the four experimental diets formulated to contain 10 or 16 % CP with 0 or 20 ppm ractopamine. Except for amino acids, additions of all other nutrients met or exceeded the NRC requirements (Table 1).

Data and sample collections

Average daily gain (ADG) average daily feed intake (ADFI) and feed efficiency (ADG/ADFI) were estimated weekly based on pig weight and feed disappearance. Blood samples were taken by venipuncture to the vena cava region at the beginning of the experiment and weekly thereafter. The samples were centrifuged at 3000 × g for 20 min. The red blood cell-free serum was extracted and maintained at -4°F until analysis for urea nitrogen concentration (SUN).

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Table 2. Response of average daily gain (ADG), average daily feed intake (ADFI), feed efficiency (ADG/ADFI), and serum urea nitrogen concentration (SUN) to 10 or 16% crude protein and 0 or 20 ppm ractopamine diets.

	Dietary protein concentration, %					P values			
	10 + 0 ppm RAC[a]	10 + 20 ppm RAC	16 + 0 ppm RAC	16 + 20 ppm RAC	SEM[b]	CP[c]	RAC	CP x RAC	SEM
Total number of pigs	9	9	9	9					
Barrows	5	5	4	4					
Gilts	4	4	5	5					
Initial weight, lb	152.80	153.1	154.66	153.42	1.95	0.68	0.76	0.68	3.83
Final weight, lb	221.26[d]	223.86[d]	241.58[e]	250.21[e]	4.295	0.01	0.24	0.55	5.99
Day 0									
SUN, mg/100 mL	8.91	8.00	8.66	10.07	1.39	0.359	0.799	0.241	0.988
Day 0 to 7									
ADG, lb	1.68[x]	1.91[x]	2.55[y]	2.48[y]	0.253	<0.0001	0.669	0.402	0.178
ADFI, lb	5.68	5.64	5.44	5.17	0.429	0.230	0.601	0.715	0.301
ADG/ADFI, lb/lb	0.29[x]	0.33[x]	0.47[y]	0.46[y]	0.037	<0.0001	0.521	0.371	0.026
SUN, mg/100 mL	11.24[x]	12.09[xy]	12.21[xy]	14.16[y]	1.39	0.129	0.158	0.577	0.988
Day 7 to 14									
ADG, lb	1.58[x]	1.99[xz]	2.26[yz]	2.60[y]	0.253	0.0004	0.040	0.790	0.178
ADFI, lb	5.75	6.38	5.77	5.81	0.330	0.258	0.163	0.222	0.235
ADG/ADFI, lb/lb	0.27[x]	0.31[x]	0.39[y]	0.45[y]	0.028	<0.0001	0.027	0.642	0.02
SUN, mg/100 mL	10.84	12.10	12.21	13.18	1.39	0.216	0.259	0.881	0.988
Day 14 to 21									
ADG, lb	1.82[xy]	1.56[x]	1.87[y]	2.29[y]	0.253	0.032	0.669	0.056	0.178
ADFI, lb	5.90[xz]	6.45[x]	5.70[z]	6.12[xz]	0.370	0.305	0.072	0.815	0.262
ADG/ADFI, lb/lb	0.30[x]	0.23[y]	0.33[xz]	0.37[z]	0.031	0.007	0.466	0.008	0.022
SUN, mg/100 mL	11.76	11.83	13.42	13.36	1.39	0.108	0.991	0.946	0.988
Day 21 to 28									
ADG, lb	1.39[x]	1.65[xy]	2.03[y]	1.98[y]	0.253	0.007	0.530	0.396	0.178
ADFI, lb	5.70	6.27	5.72	5.72	0.370	0.336	0.271	0.292	0.262
ADG/ADFI, lb/lb	0.24[x]	0.26[x]	0.35[y]	0.34[y]	0.025	<0.0001	0.806	0.307	0.018
SUN, mg/100 mL	12.14	11.60[y]	14.37[z]	13.46	1.39	0.039	0.462	0.851	0.988
Day 28 to 35									
ADG, lb	1.78[x]	1.59[x]	1.95[xy]	2.30[y]	0.253	0.015	0.667	0.134	0.178
ADFI, lb	5.97	6.10	5.57	5.92	0.399	0.320	0.396	0.684	0.281
ADG /ADFI, lb/lb	0.29[xz]	0.25[x]	0.34[xyz]	0.37[y]	0.036	0.001	0.876	0.195	0.025
SUN , mg/100 mL	11.70	11.47	14.16	12.87	1.39	0.052	0.443	0.591	0.988
Day 35 to 42									
ADG, lb	1.49[x]	1.38[x]	1.71[xy]	2.04[y]	0.253	0.014	0.545	0.220	0.178
ADFI, lb	6.63	6.34	5.94	5.99	0.623	0.252	0.792	0.703	0.449
ADG/ADFI, lb/lb	0.22	0.21	0.32	0.34	0.067	0.017	0.840	0.768	0.047
SUN, mg/100 mL	11.68	11.56	13.97	13.9	1.39	0.019	0.945	0.963	0.988
Day 0 to 42									
ADG, lb	1.62[x]	1.68[x]	2.06[y]	2.26[z]	0.129	<0.0001	0.149	0.390	0.092
ADFI, lb	5.94[xy]	6.19[x]	5.68[y]	5.79[y]	0.324	0.160	0.437	0.740	0.229
ADG/ADFI, lb/lb	0.27[x]	0.27[x]	0.37[y]	0.39[y]	0.016	<0.0001	0.416	0.279	0.011
SUN, mg/100 mL	11.18	11.24	12.71	13.01	1.23	0.067	0.842	0.893	0.872

[a]RAC = Ractopamine.
[b]SEM = Standard error of the mean.
[c]CP = Crude protein.
[d, e, x, y, z] Within a row, means without a common superscript letter differ ($P < 0.05$).

Statistical analysis

Each pig was considered an experimental unit and data were analyzed as repeated measures in time using the mixed procedure of SAS (1999). Pen was considered to be a random effect. Orthogonal contrasts were used to analyze the effects of ractopamine and CP.

Results and Discussion

There were no significant protein × ractopamine interactions except for ADG/ADFI on days 14 to 21. The overall response of ADG, ADFI, ADG/ADFI and SUN to the dietary treatments is shown in Table 2. There were dietary treatment effects ($P < 0.05$) from days 0 to 42 for ADG; where the greatest ADG was observed for pigs receiving diets with 16% CP and 20 ppm ractopamine, the lowest ADG was recorded for pigs fed the diet with 10% CP and no ractopamine; however, pigs consuming diets with low CP concentration and 20 ppm ractopamine had performance similar to pigs fed the diet with

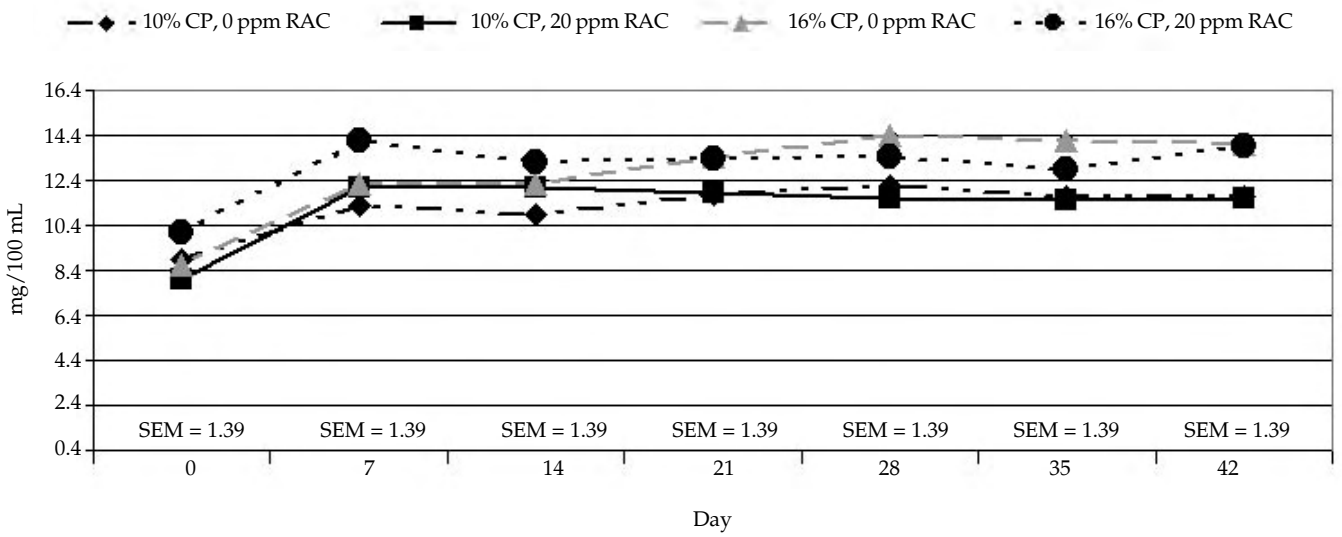

Figure 1. Response of serum urea nitrogen to experimental diets by weekly period.

0 ppm ractopamine and 10% CP. This observation was consistent for the six, seven-day periods and reflects the lack of effectiveness of ractopamine to improve ADG at low dietary CP concentrations. The addition of 20 ppm ractopamine was only effective increasing ADG when fed with a 16% CP diet (d 0-42; Table 2; $P < 0.05$). The greatest overall ADFI was for the treatment with 10% CP and 20 ppm ractopamine. There was no difference in SUN concentration among treatments.

There was no weekly or overall effect of ractopamine, except for week 2 ($P < 0.05$). The effect of ractopamine on ADG for week two is attributed to the previous observations that ractopamine increases ADG to a greater extent during the first three weeks after inclusion; however, for this study, during most periods and overall, a numeric trend showed that ractopamine inclusion resulted only in a small increase in ADG. These results demonstrate than an inadequate amino acid supply prevented pigs from responding to the inclusion of ractopamine. There was no effect of ractopamine on ADFI for period or overall experimental period; however, there was a small numerical reduction in ADFI due to increased dietary protein concentration. In contrast to literature findings, we showed a slight numerical increase in ADFI due to the inclusion of ractopamine. This trend was possibly due to the inadequate supply of amino acids, (especially lysine) needed to meet the augmented requirements for protein deposition of pigs fed diets containing ractopamine.

Increased protein concentration resulted in an improvement in ADG/ADFI for all the weeks throughout the experimental period. There was no effect of ractopamine on ADG/ADFI for all the experimental period except from days seven to 14. The inclusion of ractopamine did not improve ADG/ADFI, in contrast to responses reported by other researchers. Overall, SUN was not affected by dietary protein or ractopamine (Figure 1); however, there was an effect ($P < 0.05$) of CP on SUN on weeks 4 and 6 (Table 2). Previous reports have also shown that SUN increased when dietary CP intake increased.

Conclusions

Increasing dietary protein concentration from 10 to 16% improved growth performance when ractopamine was included at 20 ppm during the third week of the trial only; however, ractopamine showed a numerical trend to improve growth performance in every period. The 16% CP diet consistently improved growth performance (all periods and overall). Therefore, protein concentrations greater than 16% (or amino acid supplementation) are required to achieve the maximum ractopamine response in late-finishing pigs from the UNL herd.

[1]Roman Moreno is a graduate student, Robert L. Fischer is a former research technologist and graduate student, and Phillip S. Miller is a professor in the Animal Science Department.

Effect of Dietary Crude Protein Versus Crystalline Amino Acids on Growth Performance, Serum Insulin-Like Growth Factor-I Concentration, and IGF-I mRNA Expression in Growing-Finishing Gilts

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Debra K. Clopton
Andrea S. Cupp
Phillip S. Miller[1]

Summary and Implications

Fifty-six crossbred gilts with an initial body weight of 73 lb were used in a 26-day growth study. The pigs were randomly allocated to one of seven dietary treatments and individually penned (8 replicates/treatment). The dietary treatments consisted of four standard corn-soybean meal diets, which were formulated by changing the corn and soybean meal (10, 14, 18, and 22% CP) ratio and three low-protein, amino acid-supplemented diets formulated to contain similar lysine, methionine, tryptophan, and threonine concentrations as the corn-soybean meal diets (10% CP + AA,14% CP + AA, and 18% CP + AA). Pig and feeder weights were recorded weekly for the determination of ADG, ADFI, and feed efficiency (ADG/ADFI). Blood samples were collected weekly and analyzed for plasma urea and Insulin-like Growth Factor –I (IGF-I) concentrations. On day 26, real-time ultrasound backfat and longissimus muscle area measurements were recorded and used for the calculation of fat-free lean gain. There was no difference (P > 0.10) in ADFI among treatments throughout the 26-day period. Pigs fed the corn-soybean meal diets (14, 18, and 22% CP) had greater ADG (1.81 versus 1.68 lb; P < 0.05) and ADG/ADFI (0.44 versus 0.40 lb/lb; P < 0.05) than pigs fed the reduced CP amino acid-supplemented diets (10% CP + AA, 14% CP + AA, and 18% CP + AA) throughout the experiment. Fat-free lean gain increased as dietary CP or total amino acid concentration increased (P< 0.01); however, no differences (P > 0.40) were observed between gilts fed the corn-soybean meal (378 g/day) versus CP amino acid-supplemented diets (368 g/day). Increasing dietary CP or total amino acid concentration increased serum IGF-I concentrations on day 26 (P < 0.01). Serum concentration was different (P < 0.05) between gilts fed the corn-soybean meal versus low-CP, amino acid-supplemented diets (505 vs. 445 ng/mL, respectively). Real-time PCR results indicated an effect (P < 0.05) of dietary treatment on mRNA expression in the liver and semitendinosus muscle. Also, IGF-I mRNA expression was greater (P < 0.01) in the semitendinosus muscle and adipose tissue of gilts fed corn-soybean meal diets compared to gilts fed low-protein, amino acid-supplemented diets. These results suggest that the form of dietary amino acid supplementation affects serum IGF-I concentrations and mRNA expression in semitendinosus muscle and adipose tissue. The interaction between diet and the pig's growth potential are complex. The form and quantity of dietary amino acids impact this interaction. These results provide a basis to explore how diet affects the metabolic signals (e.g., IGF-I) regulating growth in the pig.

Introduction

Excessive excretion of nitrogen by livestock operations is a major environmental concern. To reduce the excretion of nitrogen from swine operations, the use of crystalline amino acids (AA) has become an important part of diet formulation within the pork industry. Crystalline AA are relatively purified sources of AA that can be added to swine diets to meet the AA requirements of pigs. The use of crystalline AA allows producers to reduce feed cost per pound of pork sold, especially during times of high soybean meal prices, and also helps producers reduce nitrogen excretion to help prevent damage to the environment. A decrease in nitrogen excretion will decrease the number of acres required for manure application. It has been estimated that for each one percentage unit reduction in CP, a 10-acre reduction will result in the land requirement for manure application for a 1,000-pig finishing operation. In addition, odors from pig manure can be offensive particularly to people not associated with agriculture, and can be a major nuisance factor. Ammonia, hydrogen sulfide, and other volatile gases that originate from the decomposition of swine manure are decreased when pigs are fed low-protein, amino acid-supplemented diets. Research indicates that for each one percentage point decrease in dietary CP there is a

Table 1. Ingredient and chemical composition of diets, as-fed basis.

	Dietary protein concentration, %						
Item	10	14	10+AA	18	14+AA	22	18+AA
Ingredient, %							
Corn	89.10	79.00	89.10	69.10	78.95	59.00	69.10
Soybean meal, 46.5% CP	5.50	15.75	5.50	25.75	15.75	36.00	25.75
Tallow	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.05	1.00	1.05	0.95	1.00	0.85	0.95
Limestone	0.70	0.65	0.70	0.58	0.65	0.55	0.58
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix [a]	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix [b]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-lysine•HCl	—	—	0.20	—	0.215	—	0.22
Threonine	—	—	0.036	—	0.036	—	0.045
Tryptophan	—	—	0.096	—	0.105	—	0.105
Methionine	—	—	0.033	—	0.033	—	0.039
Composition, %							
CP [c]	10.05	13.92	10.37	18.11	14.55	22.01	18.30
Lysine [d]	0.39	0.65	0.65	0.92	0.92	1.19	1.19
Calcium [d]	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Total phosphorus [d]	0.23	0.23	0.23	0.23	0.23	0.23	0.23
ME, Mcal/lb [d, e]	1.58	1.57	1.56	1.57	1.57	1.57	1.56

[a]Supplied per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 440 IU; α-tocopheryl acetate, 24 IU; menadione sodium bisulfite, 3.5 mg; riboflavin, 8.8 mg; d-pantothenic acid, 17.6 mg; niacin, 26.4 mg; vitamin B_{12}, 26.4 µg.
[b]Supplied per kilogram of diet: Zn (as ZnO), 128 mg; Fe (as $FeSO_4 \bullet H_2O$),128 mg; Mn (as MnO), 30 mg; Cu (as $CuSO_4 \bullet 5\ H_2O$), 11 mg; I (as $Ca(IO_3) \bullet H_2O$), 0.26 mg; Se (as Na_2SeO_3), 0.3 mg.
[c]Analyzed value.
[d]Calculated value.
[e]Metabolizable energy.

10 to 12.5% reduction in ammonia emissions.

The reduction of nitrogen excretion by pigs fed a crystalline amino acid supplemented-diet is a positive effect of feeding crystalline AA. There are however, negative effects of the reduction in CP and addition of crystalline amino acids on the rate and composition of growth in growing-finishing pigs. Many research groups have reported similar performance between pigs fed corn-soybean meal and amino acid-supplemented diets; whereas, other researchers have reported a reduction in growth performance in pigs fed amino acid- supplemented diets. A reduction in muscle protein accretion rate and an increase in fat deposition in pigs fed AA supplemented diets have been observed in some studies, whereas in other experiments no differences were detected in protein and fat accretion between corn-soybean meal and AA supplemented diets.

To date, no research has been conducted to investigate the effect of crystalline amino acids on gene expression of Insulin-like Growth Factor-I (IGF-I) and concentrations of serum IGF-I in growing-finishing pigs. The research described seeks to fill the gaps in our current knowledge of how the use of crystalline AA affects protein accretion by gaining a greater understanding of how IGF-I is affected by the dietary concentration of CP and(or) amino acids in swine growing-finishing diets. Therefore, the objective of this experiment was to investigate in vivo, the effect of increasing dietary protein and(or) crystalline AA on serum IGF-I concentration and tissue IGF-I mRNA expression in growing gilts.

Materials and Methods

Animals and Treatments

Sixty crossbred [Danbred × (Danbred × Nebraska White Line)] gilts were used in a 26-day growth study. Pigs averaged 73.0 ± 1.94 and 115.8 ± 1.36 lb at the initiation and termination of the experiment, respectively. Four gilts were randomly selected for an initial slaughter group for the collection of tissue samples. The remaining 56 gilts were randomly assigned to one of seven dietary treatments. The diets (Table 1) were standard corn-soybean meal diets or low-protein, amino acid-supplemented diets. The corn-soybean meal diets were formulated by changing the corn and soybean meal ratio and the three low-protein, amino acid-supplemented diets were formulated by reducing the CP concentration by four percentage units with the removal of soybean meal and adding back crystalline AA so that the amino acid-supplemented diets contained similar lysine, methionine, tryptophan, and threonine concentrations as the corn-soybean meal diets. The dietary treatments were 1) 10% CP diet; 2) 14% CP diet; 3) 10% CP + AA; 4) 18% CP; 5) 14% CP + AA; 6) 22% CP; and 7) 18% CP + AA. Diets were fortified with vitamins and minerals to meet or exceed

(Continued on next page)

the NRC (1998) requirements for 100-lb pigs. Pigs were housed individually and allowed ad libitum access to feed and water throughout the experiment. All experimental protocols were approved by the University of Nebraska Institutional Animal Care and Use Committee.

Data and Sample Collections

Pig and feeder weights were recorded weekly for the determination of average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (ADG/ADFI). Fat-free lean gain (FFLG) was calculated from backfat (BF) thickness and longissimus muscle area (LMA), Backfat and LMA were obtained on the first and the last day of the experiment using real-time ultrasound and fat-free lean was calculated using the National Pork Producers Council (2000) equation. Plasma and serum samples were collected weekly.

The gilts were slaughtered and organs were separated and weighed immediately after slaughter. Weights of the following organs were obtained: 1) heart with blood clots removed; 2) liver with gall bladder removed; 3) kidneys; 4) pancreas with associated fat tissue removed; 5) lungs with trachea removed; 6) stomach, which was weighed full and after contents were removed; and 7) gastrointestinal tract, which was weighed full and after contents were removed. Gastrointestinal tract was separated into small and large intestines and mesentery. Contents of the stomach and gastrointestinal tract were removed for the determination of empty body weight (live weight minus gastrointestinal content weight). Hot carcass weight was measured and the carcasses were subsequently chilled at 4ºC for 24 hours. After chilling, cold carcass weight; LMA at the tenth-rib; carcass length; tenth-rib backfat 3/4 distance along the longissimus muscle of the ribbed carcass; and midline BF depths at first-rib, 10th-rib, last-rib, and last-lumbar vertebrae were measured on each carcass. Carcasses were ground and sub samples were placed in plastic bags and frozen.

Tissue Sampling

Within 20 to 30 minutes of slaughter, tissue samples for real-time PCR were collected from different locations on the carcass. The samples collected included a liver sample from the upper left medial lobe, longissimus muscle taken at the 10th rib, semitendinosus muscle, and an abdominal subcutaneous adipose tissue sample.

Sample Analysis

Diet samples were analyzed in duplicate for DM, CP, Ca, and P. Plasma samples were analyzed for urea concentration. The concentration of IGF-I in serum was determined using a commercially available two-site immunoradiometric assay. This assay measured total serum IGF-I.

Total RNA was extracted from tissue samples using TRI-Reagent. Samples were treated with 5 units of RQ1 RNase-free DNase to remove residual genomic DNA. The 5 µg sample of total RNA was reverse transcribed into cDNA using SuperScript III reverse transcriptase.

The quantification of target cDNA coding for IGF-I and GapDH in liver, longissimus muscle, semitendinosus muscle, and adipose tissue was performed by real-time RT-PCR. The GapDH gene was chosen as a housekeeping gene and the relative concentrations of IGF-I mRNA results are expressed as the ratio IGF-I/GapDH.

Statistical Analysis

Data were analyzed as a completely randomized design using PROC MIXED of SAS. The main effect in the statistical model was dietary protein treatment and the comparison between source of amino acids, which was the comparison of corn-soybean meal diets (14, 18, and 22% CP) versus low-protein, amino acid-supplemented diets (10% CP + AA, 14% CP + AA, and 18% CP + AA.). For plasma urea and serum IGF-I concentrations the data were analyzed within week to compare the effects of dietary CP and(or) AA concentration for each week of the experiment. In all analyses, pig was the experimental unit.

Results

Growth performance. There was no difference ($P > 0.10$) in ADFI among the seven dietary treatments or between the corn-soybean meal versus the amino-acid supplemented diets throughout the 26-day experimental period (Table 2). Increasing protein concentration and amino acid concentration increased ADG, final weight, and feed efficiency ($P < 0.01$). Average daily gain increased as the dietary concentration of crude protein and(or) amino-acid supplementation increased, from 1.04 lb in gilts fed the 10% CP diet to 1.96 lb in gilts fed the 22% CP and 18% CP+AA diets ($P < 0.01$). Feed efficiency followed a similar pattern as ADG. Gilts fed the 10% dietary CP had the lowest ADG/ADFI (0.26 lb/lb) and gilts fed the diets containing 22% CP and 18% CP + AA had the greatest ADG/ADFI (0.47 lb/lb; a 55% improvement in feed efficiency; $P < 0.01$). There was a difference ($P < 0.05$) in ADG and ADG/ADFI between gilts fed the corn-soybean meal diets versus the low-protein, amino acid-supplemented diets with gilts fed the corn-soybean meal diets having greater ADG and ADG/ADFI than the gilts fed the amino acid-supplemented diets mainly due to the decrease in growth performance in gilts fed the 10% CP + AA diet.

Table 2. Effect of protein concentration and crystalline amino acids on growth performance of growing gilts.

Item		Dietary treatment								Main Effects [a]	
		10	14	10+AA	18	14+AA	22	18+AA	SEM	TRT	CP vs AA
Total number of pigs		8	8	8	8	8	8	8			
Growth performance											
	Initial wt, lb	73.18	72.54	73.18	73.05	73.43	72.76	72.85	1.940	NS	NS
	Final wt, lb	99.97	113.51	104.91	122.11	122.00	123.88	123.88	2.992	< 0.01	NS
d 0 to 26	ADG, lb [b]	1.04	1.56	1.21	1.90	1.87	1.96	1.96	0.104	< 0.01	< 0.05
	ADFI, lb [c]	3.86	4.08	3.64	4.26	4.30	4.17	4.17	0.181	NS	NS
	ADG/ADFI, lb/lb	0.26	0.39	0.33	0.45	0.44	0.47	0.47	0.012	< 0.01	< 0.05
Ultrasound measurements											
Initial	Backfat, in	0.32	0.31	0.30	0.31	0.31	0.31	0.31	0.016	NS	NS
	LMA [d], in^2	2.14	2.05	2.20	2.12	2.09	2.12	2.19	0.087	NS	NS
Final	Backfat, in	0.42	0.43	0.37	0.39	0.42	0.40	0.39	0.022	NS	NS
	LMA, in	2.62	3.16	3.45	3.72	3.52	3.70	3.79	0.128	< 0.01	NS
	FFLG [e f], g/day	183	307	301	412	379	417	425	16.8	< 0.01	NS

[a]Trt =
amino acid supplemented diets (10% CP + AA, 14% CP + AA, and 18% CP + AA), and NS = nonsignificant effect, P > 0.10.
[b]ADG = average daily gain.
[c]ADFI = average daily feed intake.
[d]LMA = longissimus muscle area.
[e]FFLG = fat-free lean gain
[f]Fat-

Carcass characteristics. At the initiation of the experiment, there were no differences (P > 0.10) in 10th-rib BF depth or LMA among the dietary treatments (Table 2). However, at the end of the experiment, there was an effect (P < 0.01) of dietary treatment on ultrasound LMA with no differences among the dietary treatments for ultrasound BF depth. Gilts fed the diets containing 18% CP, 14% CP + AA, 22% CP, and 18% CP + AA (3.72, 3.52, 3.70, 3.79 in^2, respectively) had similar LMA; however, gilts fed the 10% CP, 14% CP, and 10% CP + AA had a smaller LMA (2.62, 3.16, and 3.45 in^2, respectively). Protein and amino acid concentration had an effect (P < 0.01) on fat-free lean gain which increased as CP or total amino acid concentration increased in the diets. Gilts fed the diet containing the 18% CP + AA had the greatest accretion rate of fat-free lean (425 g/day) and gilts fed the 10% CP diet had the lowest FFLG (183 g/day) and there was no difference in FFLG in gilts fed either the corn-soybean meal (379 g/day) or the amino-acid supplemented diets (368 g/day).

Increased dietary protein concentration and total AA concentration resulted in increased hot carcass weights (P < 0.01); however, there was no difference between gilts fed corn-soybean meal versus amino acid-supplemented diets (83.46 and 83.17 lb, respectively, Table 3). Midline BF measurements and 10th-rib BF depth on ribbed carcasses were similar among the dietary treatments and no difference between corn-soybean meal versus amino acid-supplemented diets (0.30 and 0.36 in, respectively). Carcass LMA measured on the ribbed carcass at the 10th-rib increased (2.78, 3.62, 3.94, 4.24, 4.07, 4.42, and 4.26 in^2 , respectively; P < 0.01) as CP and(or) total AA concentrations increased with no difference between gilts fed corn-soybean versus amino acid- supplemented diets (4.09 versus 4.09 in^2 , respectively). Carcass length (24.54, 25.78, 24.66, 25.76, 25.66, 25.86, and 26.67 in, respectively; P < 0.05) increased as the concentration of CP and(or) total AA concentration in the diet increased and gilts fed the corn-soybean meal diets had longer carcass compared to gilts fed the low-protein, amino acid-supplemented diets (25.80 versus 25.27 in, P < 0.05). There was a trend (P < 0.10) for dressing percentage to increase as dietary CP and(or) total AA concentration increased and gilts fed the amino-acid supplemented diets (70.40%) had greater (P < 0.05) carcass dressing percentage compared to gilts fed the corn-soybean meal diets (68.71%).

Organ weights. Dietary CP and(or) total AA concentration resulted in an increase (P < 0.01) in empty body weight and there was no difference between gilts fed the corn-soybean meal versus AA supplemented diets (116.05 versus 113.97 lb; Table 4). Increased CP and(or) total AA concentration affected (P < 0.01) liver weight (1,054, 983, 895, 971, 941, 1,050, and 930 g, respectively) and gilts fed the corn-soybean meal diets had greater (P < 0.01) liver weights compared to gilts fed the AA-supplemented diets (1,001 and 922 g, respectively). Similar results were observed for kidney weight (186, 188, 167, 210, 192, 230, and 208

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Table 3. Effect of protein concentration and crystalline amino acids on carcass measuremnts of growing gilts.

	Dietary treatment								Main Effects[a]	
Item	10	14	10+AA	18	14+AA	22	18+AA	SEM	TRT	CP vs AA
Total number of pigs	8	8	8	8	8	8	8			
Carcass measurements										
Hot carcass wt, lb	67.80	77.24	74.31	86.19	86.02	86.94	89.19	2.622	< 0.01	NS
Midline backfat										
First-rib, in	1.12	1.09	1.04	1.06	1.08	1.06	1.05	0.046	NS	NS
Tenth-rib, in	0.54	0.49	0.55	0.52	0.56	0.57	0.56	0.036	NS	NS
Last-rib, in	0.50	0.47	0.47	0.46	0.49	0.52	0.48	0.035	NS	NS
Last lumbar, in	0.44	0.43	0.44	0.46	0.45	0.48	0.44	0.029	NS	NS
Other carcass measurements										
Tenth-rib, in	0.38	0.34	0.28	0.31	0.34	0.27	0.31	0.036	NS	NS
LMA[b], in^2	2.78	3.62	3.94	4.24	4.07	4.42	4.26	0.147	< 0.01	NS
Carcass length, in	24.54	25.78	24.66	25.76	25.66	25.86	26.67	0.281	< 0.05	< 0.05
Dressing %	67.80	67.27	70.00	69.50	70.49	69.36	70.71	0.943	< 0.10	< 0.05

[a]Trt =
amino acid supplemented diets (10% CP + AA, 14% CP + AA, and 18% CP + AA), and NS = nonsignificant effect, P > 0.10.
[b]LMA = longissimus muscle area.

Table 4. Effect of protein concentration and crystalline amino acids on organ weights of growing gilts.

	Dietary treatment								Main Effects [a]	
Item	10	14	10+AA	18	14+AA	22	18+AA	SEM	TRT	CP vs AA
Total number of pigs	8	8	8	8	8	8	8			
Empty body weight, lb	94.51	109.15	100.99	118.65	119.36	120.33	121.25	2.710	< 0.01	NS
Organ weights										
Heart, g	219	219	212	216	216	205	199	6.14 – 8.3	NS	NS
Liver, g	1,054	983	895	971	941	1,050	930	29.2 – 39.2	< 0.01	< 0.01
Kidney, g	186	188	167	210	192	230	208	6.3 – 8.5	< 0.01	< 0.01
Lungs, g	527	574	510	526	534	568	511	31.1 – 41.9	NS	NS
Pancreas, g	78	80	78	87	87	86	89	5.3 – 7.1	NS	NS
Stomach, g	369	367	365	327	353	342	320	9.1 – 12.3	< 0.02	NS
Small intestine, g	1,089	1,136	1,012	988	1,137	1,199	1,064	70.4 – 94.8	NS	NS
Large intestine, g	906	846	808	750	697	779	705	30.2 – 40.7	< 0.01	< 0.05
Mesentary, g	877	780	807	703	670	636	601	36.4 – 48.9	< 0.02	NS

[a]rt = co -
no acid supplemented diets (10% CP + AA, 14% CP + AA, and 18% CP + AA), and NS = nonsignificant effect, P > 0.10.

Table 5. Effect of protein concentration and crystalline amino acids on carcass accretion of growing gilts.

	Dietary treatment								Main Effects [a]	
Item	10	14	10+AA	18	14+AA	22	18+AA	SEM	TRT	CP vs AA
Total number of pigs	8	8	8	8	8	8	8			
Cold carcass weight, lb	66.64	76.18	72.50	84.87	85.55	85.44	87.87	2.434	< 0.01	NS
Accretion rates, g/day										
Protein	40	67	70	117	116	119	128	5.7	< 0.01	NS
Water	106	212	199	339	333	353	369	20.7	< 0.01	NS
Fat	175	133	149	164	182	149	169	17.6	NS	NS
Ash	10	15	13	18	18	17	16	1.1	< 0.01	NS

[a]Trt =
amino acid supplemented diets (10% CP + AA, 14% CP + AA, and 18% CP + AA), and NS = nonsignificant effect, P > 0.10.

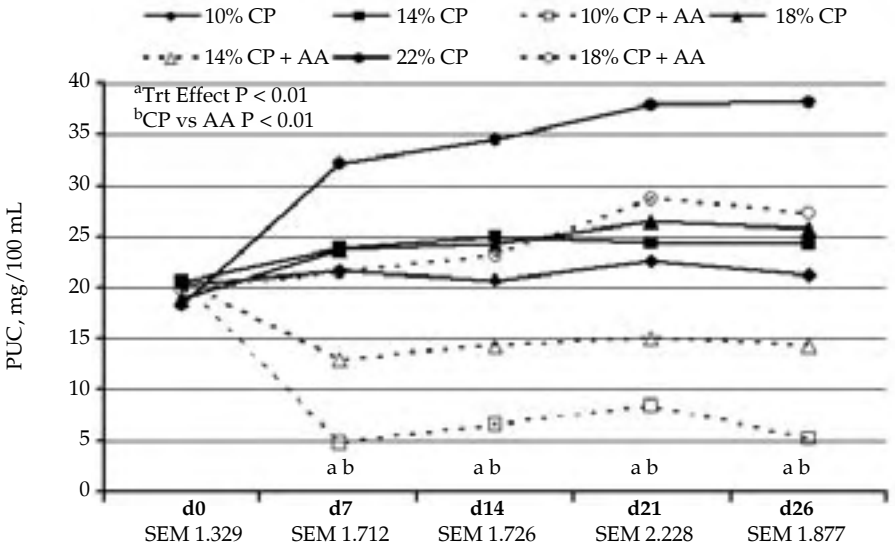

Figure 1. Response of plasma urea concentration (PUC) to experimental diets by week.

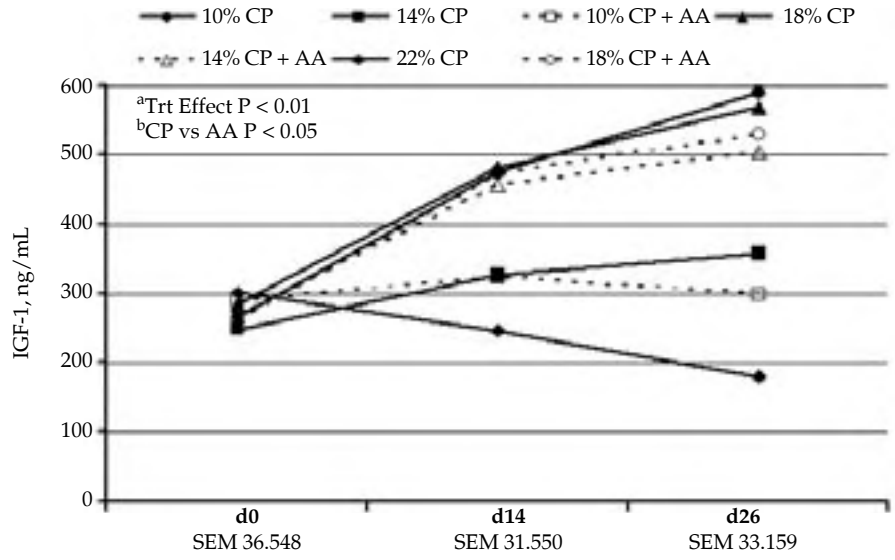

Figure 2. Response of serum insulin-like growth factor-I (IGF-I) to experimental diets by week.

g, respectively) with CP and(or) total AA concentration affecting (P < 0.01) kidney weights and gilts fed the AA-supplemented diets had lighter (P < 0.01) kidneys compared to gilts fed the corn-soybean meal diets (189 and 209 g, respectively). Stomach weight was affected (P < 0.02) by dietary treatment; however, there was no difference between gilts fed the corn-soybean versus AA-supplemented diets (345 and 346 g, respectively). Dietary treatment affected (P < 0.01) large intestinal weight and gilts fed the corn-soybean meal diets had greater (P < 0.05) large intestinal weights compared to gilts fed the AA-supplemented diets (792 and 737 g, respectively). There were no differences (P > 0.10) in the other internal organ weights (i.e., heart, lungs, spleen, small intestine, and mesentery) among dietary treatments and between corn-soybean meal versus AA-supplemented diets (Table 4).

Carcass accretion rate. Cold carcass weight increased (P < 0.01) as the concentration of dietary protein and(or) AA-supplementation concentration increased and gilts fed the corn-soybean meal and low-protein, amino acid-supplemented diets had similar (P > 0.10) cold carcass weights (82.16 and 81.98 lb, respectively, Table 5). Protein accretion rates increased (P < 0.01) from 40 g/d in gilts fed the 10% CP diet to 128 g/day in gilts fed the 18% CP + AA diet and there was no difference between gilts fed the corn-soybean meal versus the AA-supplemented diets (101 and 105 g/day, respectively). Dietary treatment increased (P < 0.01) carcass water accretion rate from 106 g/day in gilts fed the 10% CP diet to 369 g/day in gilts fed the 18% CP + AA diet and there was no difference (P < 0.10) between gilts fed the corn-soybean meal versus the AA-supplemented diets (301 and 300 g/day, respectively). Ash accretion rates increased (P < 0.01) as CP and(or) total amino acid concentration increased and there was a no difference between corn-soybean meal and AA-supplemented diets.

Blood metabolites. There was no difference among the seven dietary treatments on day 0 of the experiment; however, protein concentration and(or) AA supplementation had an effect (P < 0.01) on plasma urea concentration during week 1 thru 4 of the experiment (Figure 1). Gilts fed the amino acid-supplemented diets exhibited a decrease (P < 0.01) in plasma urea concentration compared to gilts fed the corn-soybean meal diets during week 1 through 4 of the experiment.

On day 0 there were no differences in serum IGF-I concentration among the seven dietary treatments; however, the increase in protein concentration and(or) AA supplementation resulted in an increase (P < 0.01) in serum IGF-I concentration during week 2 and 4 of the experiment (Figure 2). Gilts fed the corn-soybean meal diets had greater (505 ng/mL; P < 0.05)

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serum IGF-I concentrations compared to gilts fed the amino acid-supplemented diets (445 ng/mL) on day 26 of the experiment.

Tissue expression of mRNA. Liver IGF-I mRNA expression was affected by dietary treatment with gilts consuming the 18% CP diet having the greatest expression of IGF-I mRNA (Figure 3). Dietary CP concentration did not affect the expression of IGF-I mRNA in longissimus tissue (P = 0.20, Figure 4); however, there was an effect (P < 0.05) of dietary treatment on semitendinosus muscle. Gilts fed the corn-soybean meal diets had greater (P< 0.01) IGF-I mRNA expression in the semitendinosus muscle compared to the gilts fed the amino acid-supplemented diets (Figure 5). Insulin-like growth factor-I mRNA expression in adipose tissue was not different among the seven dietary treatments; however, gilts fed the corn-soybean meal diets had greater (P < 0.05) IGF-I mRNA expression compared to gilts fed the low- protein, amino acid-supplemented diets (Figure 6).

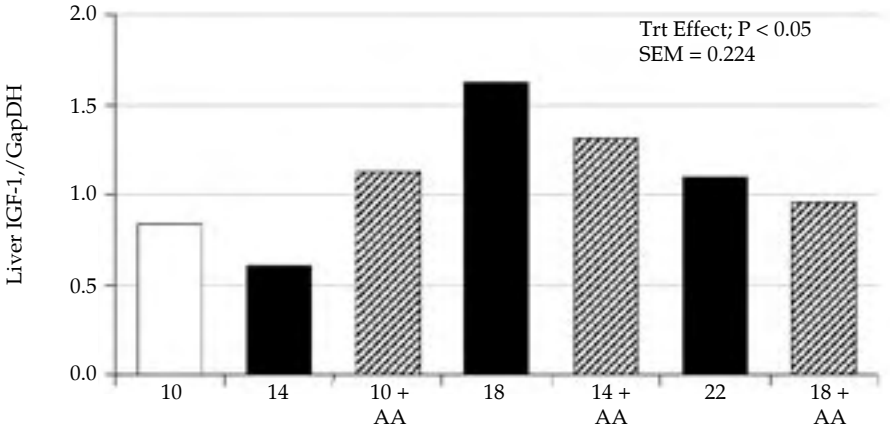

Figure 3. Effect of dietary treatment on liver IGF-I mRNA expression.

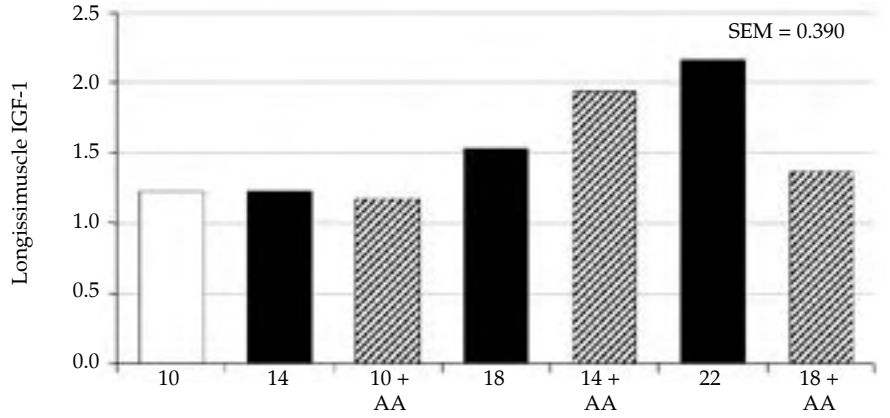

Figure 4. Effect of dietary treatment on longissimus muscle IGF-I mRNA expression.

Discussion

Previous studies investigating the effects of feeding pigs low-protein, amino acid-supplemented diets have reported a reduction in growth rate, decrease in carcass protein accretion, and increase in carcass fat accretion which could be due to a reduction in serum concentration and expression of IGF-I in various metabolic tissues. Results from this experiment do not support previous research (growth and carcass composition) because our results showed similar growth performance and carcass accretion rates in gilts fed corn-soybean meal diets compared to low-protein, amino acid-supplemented diets. However, gilts fed the corn-soybean meal diets did have greater serum IGF-I concentration and IGF-I mRNA expression in the semitendinosus muscle and adipose tissue compared to gilts fed the low-protein, amino acid supplemented diets.

Growth performance. Previous research conducted by our group showed an increase in body weight and ADG as the dietary CP concentration increased. In the current experiment, the increase in dietary CP and(or) total AA concentration resulted in an increase in ADG. Gilts fed the corn-soybean meal diets had greater ADG compared to the AA-supplemented diets. The reduced growth rate in gilts fed the 10% CP + AA diet is the main reason for the difference in ADG between gilts fed the corn-soybean meal versus AA-supplemented diets. Gilts fed the 10% CP + AA diet had similar feed intake compared to gilts fed the other diets. However, this diet was formulated to contain total amino acid concentrations below the gilts' requirements. The ratio of lysine to isoluceine and valine was lower than in the 14% CP diet and these lower AA ratios possibly caused the reduction in ADG. Gilts fed the 18% CP and 14% CP + AA diets and the 22% CP and 18% CP + AA had similar growth performance throughout the experiment. Feed intake was not different among the seven dietary treatments. Feed efficiency (ADG/ADFI) increased as a result of the increase in the dietary

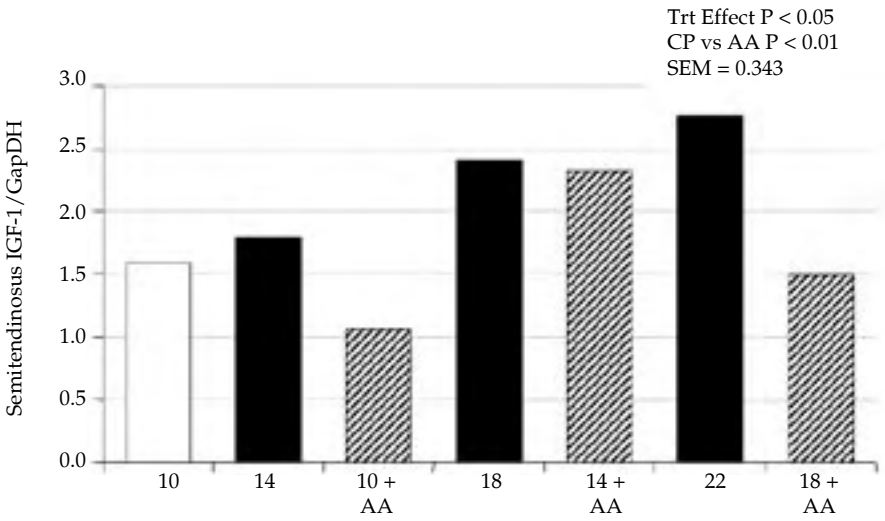

Figure 5. Effect of dietary treatment on semitendinosus muscle IGF-I mRNA expression.

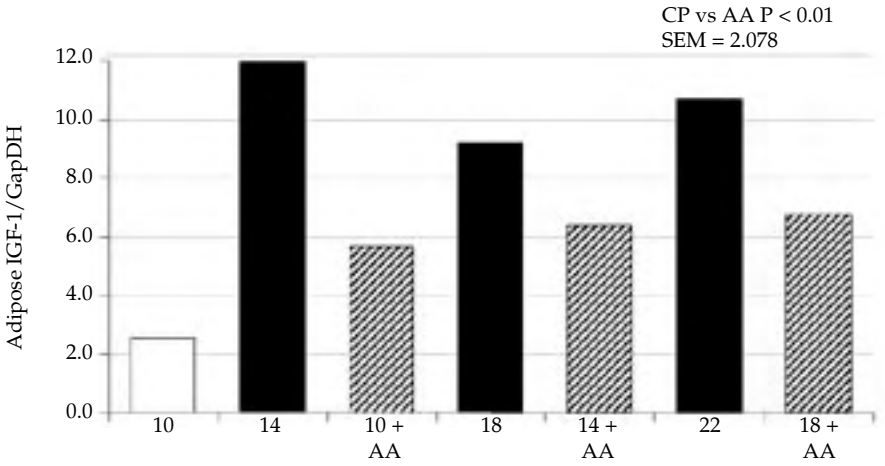

Figure 6. Effect of dietary treatment on subcutaneous adipose tissue IGF-I mRNA expression.

concentration of CP and(or) total AA. These results are similar to those from previous experiments; however, in this experiment, gilts fed the AA-supplemented diets exhibited a reduction in feed efficiency as compared to those fed the corn-soybean meal diets. The difference in feed efficiency between corn-soybean meal and AA-supplemented diets can again be attributed to the reduction in ADG observed in gilts fed the 10% + AA diet. Gilts fed the 18% CP, 14% CP + AA, 22% CP, and 18% CP + AA had numerically similar estimates for feed efficiency (0.45, 0.44, 0.47, and 0.47 lb/lb, respectively).

Carcass measurements. Ultrasound measurements taken on d 0 and 26 of the experiment showed no difference in BF depth among the seven dietary treatments or between gilts fed corn-soybean meal versus AA-supplemented diets. Also, carcass measurements taken 24-h after slaughter indicated no difference in 10th-rib BF depth among the dietary treatments or between corn-soybean meal and amino acid-supplemented diets. Carcass LMA increased as dietary CP and(or) AA concentration increased, but no differences were detected between corn-soybean meal and AA-supplemented diets. Using the National Pork Producers Council equations, FFLG increased as dietary CP and(or) dietary AA concentration increased from 183 g/day in gilts fed the 10% CP diet to 425 g/day in gilts fed the 18% CP + AA. Again, there was no difference between corn-soybean meal and AA-supplemented diets. The increase in LMA is not surprising because as the dietary CP intake increased from below the requirement, the concentration of AA available for muscle protein accretion increased.

Organ weights and carcass accretion rates. Empty body weight increased as CP and(or) AA concentration increased and there was no difference between gilts fed the corn-soybean meal and AA-supplemented diets. The reduction in dietary protein concentration in the AA-supplemented diets caused a reduction in liver and kidney weight because of the decrease in amino acids that must be processed by the liver and cleared from the body via the kidneys in the form of urea nitrogen. The decrease in stomach weight as dietary protein and(or) AA concentration increased is interesting in that there was no difference in ADFI. Cold carcass weight was increased as CP and(or) AA intake increased from below the requirements and no difference was observed in cold carcass weight between gilts fed the corn-soybean meal or AA-supplemented diets. The heavier cold carcass weight in pigs consuming a diet with a greater percentage of CP and(or) AA is supported by the increase in ADG.

Blood metabolites. Plasma urea concentrations were reduced when gilts were fed the low-protein, AA-supplemented diets compared to corn-soybean meal diets. An increase in plasma urea concentration is a metabolic response to the increase in protein intake once amino acid requirements are met or amino acid imbalances are created. As dietary protein intake

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increases above the requirement for protein accretion, the excess amino acids must be processed in the liver by deaminating the excess amino acids and removing the nitrogen by producing urea which is filtered out of the blood by the kidneys and excreted from the body in urine. Thus, feeding low-protein, AA-supplemented diets reduces the excess non-essential AA that are not used for protein deposition, and thus the nitrogen (urea) excreted is reduced. The increase in plasma urea concentration in the pigs fed the 22% CP diet indicates that the dietary CP requirement for pigs in this experiment was greater than 18% CP and is supported by the NRC (1998) model which suggests that the dietary CP requirement for high-lean gain gilts with a body weight of approximately 100 lb is 19.2% CP.

Gilts fed the 10% + AA and 14% CP had similar serum IGF-I concentrations and had IGF-I concentrations greater than gilts fed the 10% CP diet which had the lowest serum IGF-I concentration. The IGF-I concentrations increased from day 0 to day 14, and to day 26 of the experiment indicating that serum IGF-I concentration responded quickly to a change in dietary CP concentration. However, it was interesting to detect a difference in serum IGF-I concentration between the sources of amino acids, with gilts fed the corn-soybean meal diets having greater serum IGF-I concentrations as compared to the gilts fed the low-protein, AA-supplemented diet. Results from this experiment indicate that the production and release of IGF-I into the blood is inhibited by the consumption of a diet providing AA concentrations below the requirements (10% CP, 10% CP + AA, and 14% CP diets). This reduction in serum IGF-I is a possible causative factor in the reduction in FFLG and carcass protein accretion rates in the gilts consuming the 10% CP, 10% CP + AA, and 14% CP diets. These results suggest that the consumption of a diet deficient in CP and(or) amino acids does inhibit the production of IGF-I and the actions of IGF-I (i.e., muscle protein accretion) are partially inhibited.

Tissue IGF-I Expression. Expression of IGF-I mRNA in the longissimus muscle was not affected by dietary protein and(or) amino acid concentration. However, there was a significant increase in IGF-I mRNA in liver and semitendinosus muscle tissue. The data from the current experiment suggest that the circulating concentration of IGF-I does have an effect on muscle growth and that the actions of IGF-I on muscle may function in both an endocrine and autocrine/paracrine manner. This statement is supported by data from the current experiment that showed both a decrease in serum IGF-I concentration and reduced tissue mRNA expression in gilts fed diets not meeting amino acid requirements.

Conclusion

Results from this experiment demonstrate that growing gilts respond to increased dietary CP and(or) amino acid concentrations. As dietary CP and amino acid concentrations were increased in the diet from deficient to adequate concentrations there was an improvement in ADG, feed efficiency, and FFLG. A similar effect was detected in plasma urea concentrations. Pigs fed the 22% CP diets had an increase concentration of plasma urea compared to the pigs fed the 10, 14, and 18% CP diets. Gilts fed the 18% CP + AA had greater plasma urea concentration than gilts fed the 10% CP + AA and 14% CP + AA, indicating that the CP requirement of gilts in this experiment was > 18% CP and 0.92% total lysine. However, serum IGF-I concentrations were decreased in pigs fed the 10% CP, 10% CP + AA and 14% CP diets, indicating that the consumption of a diet below the pigs dietary crude protein requirement (18%) was associated with a reduction in IGF-I serum concentration. Also, serum IGF-I concentrations were reduced in gilts fed the low-protein, AA-supplemented diets which is supported by the reduction in IGF-I mRNA in semitendinosus and adipose tissue. However, this reduction in IGF-I serum concentrations or mRNA expression did not result in reduction in FFLG or carcass protein accretion rate. Thus, the reduction in serum IGF-I concentration in gilts fed the AA-supplemented diet was not severe enough to have an impact on lean growth rate or carcass protein accretion. Therefore, the results of the current experiment suggest that the feeding of low-protein, AA-supplemented diets does result in a decrease in serum IGF-I concentration and IGF-I mRNA expression in semitendinosus and adipose tissue, but this reduction in expression and serum concentration only partially explains growth rate and carcass composition results for gilts fed corn-soybean meal and low-protein, AA-supplemented diets.

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Genes Expressed in Response to PRRSV

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Summary and Implications

Gene maps of livestock are rapidly being developed and have led to an explosion of knowledge in recent years about genes affecting economic traits. One potential application of this information that would have major economic value is in selection of livestock for resistance to disease. Even though much has been learned about Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) since it was first identified, PRRSV continues to cause significant economic losses in many herds. Traditional approaches to manage PRRSV can be effective, but may be costly and have not always resulted in permanent control. This is a disease for which application of molecular genetic knowledge to select for resistance would have significant economic advantages. An experiment was initiated at Nebraska to investigate possible genetic variation among pigs in response to PRRSV. Pigs from two populations were infected with PRRSV, responses over 14 days were recorded, and tissues were collected at necropsy for gene expression studies. Phenotypic data, including body weights and rectal temperatures, viremia, and lung lesion scores, provided substantial evidence that genetic variation in response to PRRSV exists. With that knowledge, we developed an index of high (H) and low (L) responders, indicating susceptible and resistant phenotypes, and measured expression differences in lung and bronchial lymph node of 11 immune function genes between H and L pigs. Ten of these genes, involving both innate and acquired immune function, were expressed differently in lung and/or lymph tissue. They tended to be up-regulated (expressed at greater levels) in H pigs. We demonstrated that genetic variation in response to PRRSV exists and that both innate and acquired genes are involved. We have not yet determined whether selection for the immune function genes involved or levels of the proteins they produce will be effective in selecting for PRRSV resistance. Results will be helpful in additional investigations aimed at developing methods to select for resistance to PRRSV.

Introduction

The National Pork Board has estimated that disease due to the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes annual losses of approximately $560 million to U.S. pork producers. To combat PRRSV, the Coordinated Agricultural Program (CAP) grant awarded to researchers in the industry by USDA National Research Initiative (NRI), a major, multi-disciplinary initiative to eradicate PRRSV, was implemented.

One objective of the NRI CAP grant is to investigate the possibility of genetically modifying the pig as a means to control PRRSV. The hypothesis is that genetic variation in response to PRRSV exists, that the genes responsible for this variation can be identified, and that selection on these genes will be effective in developing lines of pigs resistant to PRRSV. An experiment was initiated at the University of Nebraska to test this hypothesis. The objectives were 1) to determine whether genetic variation exists, 2) to identify traits that differentiate animals that respond differently to PRRSV, 3) to build the phenotypic and genotypic records to quantify genetic variation between animals and 4) to identify the genes involved in the response.

Nebraska PRRSV Infection Experiment

The description of the experiment and biological responses relating to objectives 1 to 3 were reported in the *2004 Nebraska Swine Report.* An overview is included here.

Pigs of each of two populations were infected with PRRSV and their phenotypic responses over a 14-day period were recorded. A total of 200 pigs from the Nebraska Index line (I), a Large White-Landrace composite population that has been selected for increased litter size for 20 generations, and 200 pigs from a cross of Hampshire and Duroc lines (HD) that have been selected for rate and efficiency of lean growth were used. Line I pigs were born in the University of Nebraska swine research herd, whereas HD pigs were obtained from a commercial farm. Neither farm had experienced disease from PRRSV and pigs from both herds had tested negative for the presence of PRRSV by PCR methods and for PRRSV serum antibodies by the ELISA test. Pigs represented a total of 83 sires and 163 dams.

Pigs were transported at an average age of 23 days to the University of Nebraska Veterinary and Biomedical Sciences (VBS) Animal Research Facility and placed in environmentally controlled rooms, two pens per room, and 12 to 13 pigs per pen. One room was randomly assigned for treatment and the pigs were inoculated intranasally with 2 ml (1 ml per nostril) of 10^5 $CCID_{50}$ (cell culture infectious dose 50% per ml) of PRRSV strain 97-7985 supplied by Dr. Fernando Osorio. Pigs in the other isolated room were littermates to the infected pigs and served as controls. Data were collected on days 0, 4, 7 and 14 post-inoculation. Pigs

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were killed at day14, necropsy was performed, and samples of several tissues were colleted for gene expression experiments.

Phenotypic Traits Measured

Body temperature by rectal probe, body weight, and blood draws were collected and recorded just before inoculation (day 0) and 4, 7, and 14 days after inoculation. At necropsy, samples of lung, bronchial lymph node, and spleen were collected and stored at -80ºC. Viremia ($CCID_{50}$/ml), a measure of each pig's ability to replicate the virus, was measured in serum collected at days 4, 7, and 14, and in lung and bronchial lymph node collected at necropsy. An ELISA test was conducted on serum samples collected at d14 to determine the level of PPRSV antibody in infected pigs and to test for possible cross contamination in uninfected pigs.

Lungs were scored for the presence of pneumonia (yes (1) or no (0)). Sections of lung were examined by light microscopy and scored on a scale of 1 to 3. Lungs receiving a score of 1 had no lesions or had mild multifocal interstitial pneumonia. A score of 2 was given to lung sections that had moderate interstitial pneumonia involving less than 50% of the area of the section. A score of 3 was given to lung sections that had greater than 50% involvement of severe interstitial pneumonia. If present, lesions suggestive of *mycoplasma hyopneumonae* and infectious bacterial pneumonia were recorded.

Identifying resistant and susceptible pigs

Principal component (PC) analysis, a multivariate procedure, was used to identify pigs in the outermost tails of the distribution of response variables. This procedure combined all variables into an index in which traits were weighted according to their contribution to total variation. Traits included were viremia at 4, 7, and 14 days post-infection (dpi), weight change from 0 to 4, 4 to 7, and 7 to 14 dpi, rectal temperature change from 0 to 4, 4 to 7, and 7 to 14 dpi, lung and bronchial lymph node viremia, and severity of lung lesions. Pigs receiving high PC values had high viremia and high symptoms of PRRSV (H) and pigs with low PC values had low viremia and low symptoms (L). Based on the PC analysis, 7 H and 7 L pigs in each population and their uninfected littermates (total of 56 pigs) were used in a 2*2*2 factorial design that included class (pigs classed as H or L responders), line (I or HD), and treatment (infected or uninfected).

Gene expression analyses

RNA from the lung and bronchial lymph node (BLN) tissues of the 56 pigs was extracted and gene expression was evaluated with RT-PCR. Gene expression cycle thresholds (Ct), which are directly related to the initial amount of target DNA present, were recorded. A low Ct value means that the expression level of that gene was high because fewer PCR cycles were required to reach the threshold, and vice versa, a high Ct value means that the expression level was low because it took more cycles to reach the threshold. Thus, samples producing low Ct values had more cDNA, indicating greater expression of the gene, than those with high values.

Five genes with innate immune function, five genes with acquired immune function, one regulator gene, and a housekeeping gene to monitor experimental procedures were evaluated. Innate immunity is the basic resistance to disease that an animal possesses. It is the first line of defense against infection. Responses are immediate and broad-spectrum, without memory, so there is no lasting protective immunity. Acquired immunity on the other hand occurs in response to infection and is often called adaptive, as the immune system must adapt itself to previously unseen molecules. Responses are slower, but in the case of certain organisms, this type of immunity can be long-lasting as the individual may be immunized against certain organisms.

Genes involved in Innate Immunity

- Interferon alpha (IFNA) – induces an antiviral response or resistance to viral replication by binding to the interferon □/□ receptor. Once bound, IFNA helps activate the JAK-STAT pathway, which in turn induces the transcription of several genes. Genes activated by IFNA contribute to the inhibition of viral replication. The IFNA gene is on chromosome 4.
- Interleukin 1 beta (IL1B) - Produced by activated macrophages, IL-1 stimulates thymocyte proliferation by inducting IL-2 release, B-cell maturation and proliferation and fibroblast growth factor activity. The IL1B gene is on chromosome 3.
- Interleukin 6 (IL6) - is a cytokine with a wide variety of biological functions: it plays an essential role in the final differentiation of B-cells into IG-secreting cells; it induces myeloma and plasmacytoma growth, nerve cell differentiation in hepatocytes, and acute phase reactants. IL6 is located on chromosome 9.
- Interleukin 8 (IL8) - is a chemotactic factor that attracts neutrophils, basophils and T-cells but not monocytes. It is also involved in neutrophil activation and is released from several cell types in response to an inflammatory response. IL8 is located on chromosome 8.
- Colony stimulating factor 2 (CSF2) – named for their ability to induce the formation of distinct hematopoietic cell lines. CSF2 is located on chromosome 5.

Genes involved in Th1 (acquired) immune response

- Interferon gamma (IFNG) - produced by lymphocytes and activated by specific antigens or

mitogens. IFN-gamma, in addition to having antiviral activity, has important immunoregulatory functions. It is a potent activator of macrophages, and has anti-proliferative effects on transformed cells and it can potentiate the antiviral and anti-tumor effects of the type I interferons. IFNG is located on chromosome 5.

• Interleukin 12 beta (IL12B) – functions to stimulate the synthesis of interferon-gamma by T-lymphocytes and NK cells; increases the killing activity of CTLs and NK cells; and stimulates the differentiation of naive T4-lymphocytes into interferon-gamma producing Th1 cells. It is produced mainly by macrophages and dendritic cells. IL12B is located on chromosome 5.

• Interleukin 15 (IL15) – stimulates NK cell proliferation and proliferation of T-lymphocytes. IL-15 is produced by various cells including macrophages. IL15 is located on chromosome 8.

• Signal transducer and activator of transcription 1-alpha (STAT1) – binds to phosphorylated tyrosine residues playing an essential role in the signaling pathways of a variety of cytokines. STAT1 is located on chromosome 15.

• Tumor necrosis factor (TNF) – it is a cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. It is a potent pyrogen causing fever by direct action or by stimulation of interleukin 1 secretion and is implicated in the induction of cachexia. Under certain conditions it can stimulate cell proliferation and induce cell differentiation. TNF is located on chromosome 7.

Genes involved in the T-regulatory immune function

• Interleukin 10 (IL10) – stimulates proliferation of B cells, thymocytes, and mast cells. It also antagonizes generation of the Th1 subset of helper T cells so it is an inhibitor of activated macrophages and dendritic cells. As such, it regulates innate immunity and cell-mediated immunity. IL10 is located on chromosome 1.

Housekeeping gene

• Ribosomal protein L32 (RPL32) – ubiquitous protein found throughout the body. This gene is used to determine if protocols were working properly because all pigs should have approximately the same level of the protein, however levels may differ across tissues. RPL32 is found on several chromosomes.

Results

There was substantial evidence for genetic variation as pigs of the two populations had distinctly different responses to PRRSV (see the 2004 Nebraska Swine Report). With that evidence in hand, we developed the Principal Component Index to describe susceptible and resistant pigs. The seven highest and lowest responders in each population and their littermates were used to determine whether specific immune function genes were expressed differently in tissues of these pigs.

Table 1 contains the overall line means for individual traits and the means of the seven pigs in each line classified as susceptible (H, high viremia and high symptoms of PRRSV) or resistant (L, low viremia and low symptoms). All pigs replicated PRRSV as evidenced by elevated serum viremia values at day 4. Serum viremia of L class pigs in Line I dropped sharply at day 7 and declined to values near zero by day 14, whereas serum viremia of H pigs remained high throughout. In both lines, L pigs showed immediate symptoms of PRRSV with slightly decreased weight gain and increased temperature, but they recovered quickly and gained weight at near normal rates and had near normal temperatures from day 7 to 14, whereas H pigs had high temperatures, with the exception of temperature of HD pigs, from day 7-14, and low or negative weight gain during each period. The general nature of the response was similar in both lines, but the difference between H and L pigs was greater in I than in HD, suggesting resistant I pigs (the L class) had a stronger innate immune response to PRRSV than HD pigs. This difference in response between lines was supported by the gene expression data (see below).

Differences in gene expression levels in lung and bronchial lymph nodes between infected and uninfected pigs, between susceptible (H) and resistant (L) pigs that had been infected with PRRSV, and between uninfected littermates of H and L pigs are in Table 2. Gene expression is measured by the number of cycles to produce a predefined threshold level of cDNA, the complementary DNA of the RNA harvested from the tissue. Each cycle doubles the amount from the previous cycle. Therefore, differences of 1 Ct represent a two-fold difference in cDNA, differences of 2 represent a four-fold difference, etc.

The first contrast (I – UI) contrasts gene expression between infected (+) and uninfected (-) pigs. It shows which immune function genes were stimulated to produce RNA in response to PRRSV. Seven of the 11 genes studied responded in lung tissue ($P < 0.05$) and all were up-regulated as it took fewer Ct cycles to produce the threshold level of cDNA. Three genes (IL1B, IL8, and CSF2) are innate immunity genes, and four (IFNG, IL12B, STAT1, and TNF) are part of the acquired immune response. Each of these genes except IL1B also was up-regulated in bronchial lymph nodes. Three additional genes also were up-regulated in lymph tissue (INFA, IL10, and IL6). Therefore, every gene studied except IL15, including the regulator gene IL10, was expressed in greater amounts in either or both lung and lymph

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tissue in response to PRRSV.

The next contrasts (H - L) are between pigs classed as susceptible (H) and resistant (L). It was done both in infected (+) and in uninfected littermates (-) to determine whether resistance occurred because pigs had a greater capacity to respond to the virus (measured in infected pigs), or whether there was naturally greater expression of the gene even in the absence of virus (measured in uninfected littermates).

Susceptible pigs (H class) had greater expression of four genes in both lung and lymph. Two of these genes are involved in innate immunity (IL1B and IL8), INFG is involved with acquired immunity, and IL10 is a regulator gene. An additional acquired immunity gene, STAT1, had greater expression in lung. In all cases, resistant pigs (L class) had less expression of these genes. For two of these genes (INFG and IL10), uninfected littermates of resistant pigs also had lower expression levels than uninfected susceptible pigs. This suggests that the expression of these genes may be an inherent difference between H and L pigs that exists without the stimulus of PRRSV. There were no similar cases in lymph tissue. Therefore, most of the differences in gene expression between susceptible and resistant pigs probably occurred as a response to PRRSV.

Table 1. Overall line means and means for the seven high (H) and low (L) responders within each line based on Principal Component Index.

	Line I			Line HD		
Item	Overall[a]	H[b]	L[c]	Overall[a]	H[b]	L[c]
			Viremia, log_{10} $CCID_{50}$/ml			
Serum, day 4	4.17	4.39	4.11	4.54	5.11	3.10
Serum, day 7	3.91	4.47	3.20	4.40	5.13	3.64
Serum, day 14	3.00	4.49	0.50	3.59	5.29	2.51
Lung	3.96	5.07	2.40	4.45	4.71	4.21
Lymph	2.55	3.33	1.31	3.12	3.70	2.66
			Body weight change, lb			
Day 0 to 4	0.71	0.18	1.28	0.64	-0.09	1.17
Day 4 to 7	0.73	-0.02	2.03	0.13	-0.40	0.82
Day 7 to 14	2.98	0.68	4.87	1.57	-1.26	3.97
			Rectal temperature change, °F			
Day 0 to 4	1.66	1.96	.59	3.17	1.42	3.49
Day 4 to 7	1.04	1.80	1.19	1.48	2.45	-0.11
Day 7 to 14	-0.30	0.65	-1.81	-0.65	-5.11	-0.47
			Lung lesion score			
	1.26	1.57	1.00	1.96	1.57	2.00

[a]Overall line mean (n = 100).
[b]Mean of 7 pigs classed as high responders based on principal component index.
[c]Mean of 7 pigs classed as low responders based on principal component index.

Table 2. Differences in gene expression levels (Ct values) between infected (I) and uninfected (UI) pigs, between infected susceptible (H^+, high responders) and infected resistant (L^+, low responders), and between uninfected littermates (-) of H and L class pigs.

	Lung			Bronchial lymph node		
Gene	I - UI	$H^+ - L^+$	$H^- - L^-$	I - UI	$H^+ - L^+$	$H^- - L^-$
IFNA	-0.03	-0.88	-0.71	-0.53**	-0.60	-0.48
IFNG	-2.15**	-2.40**	-1.36**	-2.18**	-1.43**	0.25
IL15	-0.12	-1.51	-0.76	-0.15	-1.73	-0.82
STAT1	-0.82**	-1.59*	-0.88	-0.82**	-0.56	0.00
IL1B	-2.03**	-1.38*	0.21	-0.38	-1.66**	0.89
IL12B	-1.22**	-0.31	1.02	-0.69*	-0.16	0.66
CSF2	-1.10**	-0.71	0.28	-0.73**	0.23	0.48
IL8	-2.26**	-1.80*	-0.24	-2.02**	-2.17*	0.98
IL10	-0.43	-2.53**	-1.75**	-0.68*	-1.76*	-0.42
IL6	-0.06	-0.81	-0.50	-0.56*	-0.60	0.14
TNF	-0.68*	-1.36	-0.44	-0.67**	-0.08	0.64

Interactions

Only a few interactions of class (resistant vs susceptible) with genetic line existed, indicating that the general nature of expression differences between H and L pigs was consistent across lines. However, several significant interactions of genetic line with treatment (infected vs uninfected) existed. Interaction existed ($P < 0.05$) for expression of IFNG, IL12B, Il8, and TNF in lung, and for IFNA in lymph node. The general nature of these suggested that Line I had a greater innate response to PRRSV whereas Line HD had a greater acquired response.

As an example of these interactions, expression of the innate gene IFNA in lymph was nearly identical in infected and uninfected I pigs (30.3 vs 30.4 Ct), whereas the difference was more than two-fold between infected and uninfected HD pigs (30.5 vs 29.3). Infection reduced expression of INFA in HD, but had no effect in I pigs.

Expression of the IFNG gene involved in acquired immunity is another example. The Ct values for uninfected and infected I pigs were 27.3 and 26.2, respectively, whereas values for uninfected and infected HD pigs were 28.1 and 24.9, respectively. Infection stimulated a significantly greater expression of IFNG in HD pigs.

In general these interactions supported the suggestion from the phenotypic data that I-pigs had a stronger innate response to PRRSV, whereas HD pigs had a stronger acquired response. All pigs exhibited symptoms of PRRSV, but due to greater innate immunity, I pigs recovered more quickly and had greater weight gain and lower rectal temperatures, especially from day 7 to 14, than HD pigs.

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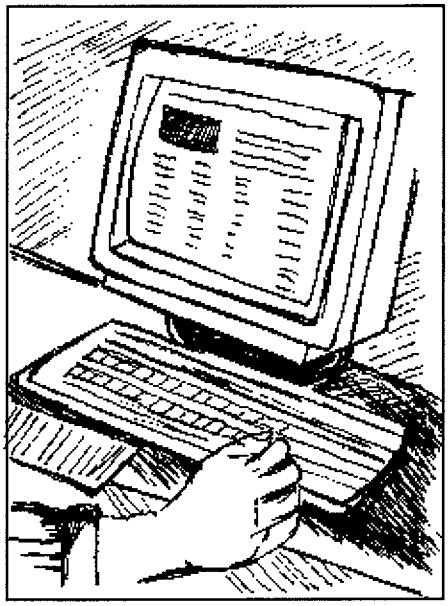
Explanation of Statistics Used in This Report

Pigs treated alike vary in performance due to their different genetic makeup and to environmental effect we cannot completely control. When a group of pigs is randomly allotted to treatments it is nearly impossible to get an "equal" group of pigs on each treatment. The natural variability among pigs and the number of pigs per treatment determine the expected variation among treatment groups due to random sampling.

At the end of an experiment, the experimenter must decide whether observed treatment differences are due to "real" effects of the treatments or to random differences due to the sample of pigs assigned to each treatment. Statistics are a tool used to aid in this decision. They are used to calculate the probability that observed differences between treatments were caused by the luck of the draw when pigs were assigned to treatments. The lower this probability, the greater confidence we have that "real" treatment effects exist. In fact when this probability is less than .05 (denoted **P < .05** in the articles), there is less than a 5% chance (less than 1 in 20) that observed treatment differences were due to random sampling. The conclusion then is that the treatment effects are "real" and caused different performance for pigs on each treatment. But bear in mind that if the experimenter obtained this result in each of 100 experiments, 5 differences would be declared to be "real" when they were really due to chance. Sometimes the probability value calculated from a statistical analysis is P < .01. Now the chance that random sampling of pigs caused observed treatment differences is less than 1 in 100. Evidence for real treatment differences is very strong.



It is commonplace to say differences are significant when P <.05, and highly significant when P < .01. However, P values can range anywhere between 0 and 1. Some researchers say that there is a tendency that real treatment differences exist when the value of P is between .05 and .10. Tendency is used because we are not as confident that differences are real. The chance that random sampling caused the observed differences is between 1 in 10 and 1 in 20.

Sometimes researchers report **standard errors of means (SEM)** or **standard errors (SE)**. These are calculated from the measure of variability and the number of pigs in the treatment. A treatment mean may be given as 11 ± .8. The 11 is the mean and the .8 is the SEM. The SEM or SE is added and subtracted from the treatment mean to give a range. If the same treatments were applied to an unlimited number of animals the probability is .68 (1 = complete certainty) that their mean would be in this range. In the example the range is 10.2 to 11.8.

Some researchers report **linear (L)** and **quadratic (Q)** responses to treatments. These effects are tested when the experimenter used increasing increments of a factor as treatments. Examples are increasing amounts of dietary lysine or energy, or increasing ages or weights when measurements are made. The L and Q terms describe the shape of a line drawn to describe treatment means. A straight line is linear and a curved line is quadratic. For example, if finishing pigs were fed diets containing .6, .7, and .8% lysine gained 1.6, 1.8 and 2.0 lb/day, respectively we would describe the response to lysine as linear. In contrast, if the daily gains were 1.6, 1.8, and 1.8 lb/day the response to increasing dietary lysine would be quadratic. Probabilities for tests of these effects have the same interpretation as described above. Probabilities always measure the chance that random sampling caused the observed response. Therefore, if P < .01 for the Q effect was found, there is less than a 1 % chance that random differences between pigs on the treatments caused the observed response.

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