

# NEBRASKA SWINE REPORT

- **Nutrition**
- **Genetics**
- **Health**



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Prepared by the staff in Animal Science Department and cooperating departments for use in Extension, Teaching, and Research programs.

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### 2011 Nebraska Swine Report

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## Rodger Johnson Retires

Patience and perseverance have guided a career in swine genetics.

For anyone interested in learning more about the genetic components of litter size and how they affect reproductive efficiency in swine, the road to that knowledge will likely lead through the East Campus of the University of Nebraska–Lincoln (UNL). It is there that swine geneticist Rodger Johnson has spent over 30 years teaching animal breeding and researching the genetic and physiological components of sow productivity and, more recently, the genetics of disease resistance.

Johnson grew up on a 1,250 acre, predominantly grain and cattle farm in northwest North Dakota where “pigs were never a big deal,” he remembers. With two brothers and four sisters, what was a big deal was making sure he and his siblings pursued a higher education.

When he graduated from high school in 1961, a drought-stricken wheat crop tapped the Johnson family dearly, but a local banker’s advice echoed in his father’s ears. “You’ve got to find a way to educate those kids; it’s the single most important thing you can do,” he urged.

“Dad told me — get a job, go to college, find a way to get through. If I have any money to help you, I will, but I can’t promise anything,” Johnson recalls.

Parental lessons in hard work and commitment took hold and inspired Johnson to pursue a Bachelor of Science degree in animal science at North Dakota State University (NDSU). Within two months after receiving his degree in 1965, Uncle Sam drafted him for a two-year hitch in the Army.

Returning from Vietnam in 1967, Johnson took an assistant county agent position in Barnes County, North Dakota, where the focus was crops and cattle. One day his NDSU undergraduate advisor, Merle White, contacted him with news that a graduate level assistantship was available at Oklahoma State University (OSU), and he nudged Johnson to take a look.

“I think Merle recognized something in me that I probably didn’t recognize in myself,” Johnson explains. The catch was, Joe Whiteman, chairman of the animal science graduate committee at OSU, insisted on a face-to-face meeting with all applicants.

Johnson made the trek to Stillwater, secured the assistantship and spent the next couple of years studying carcass composition in beef cattle. As part of the master’s degree program, Johnson took an advanced animal breeding course, which introduced him to what would become a career in swine genetics, and a person who would become a treasured mentor throughout his academic career — OSU swine geneticist Irv Omtvedt.

Johnson completed the master’s degree program in 1971, and stayed on to get a Ph.D. Despite a slight preference for cattle, Johnson was drawn to Dr. Omtvedt’s



Photo courtesy of National Hog Farmer

reputation as an excellent teacher, advisor, and mentor. “I chose the person to work with, rather than the species to work with,” he explains.

From that point forward, the swine genetics die that has carried Johnson through nearly 40 years of teaching animal breeding and swine-based research was cast.

### Crossbreeding Trials

At OSU, Omtvedt had set up a swine crossbreeding research program designed to help commercial producers understand the merits of crossbreeding. “There were a lot of questions about the strengths and weaknesses of the pure breeds and how they combined together,” Johnson explains.

Duroc, Hampshire, and Yorkshire pure lines were evaluated in all possible combinations, using them as sire lines and replacement gilt/maternal lines, to gain estimates of heterosis (hybrid vigor), and to identify which combinations performed best. Results were the focus of Johnson’s Ph.D. dissertation.

Soon after, Omtvedt left to take an associate dean position at Auburn University, leaving a vacancy that he recommended Johnson fill.

Johnson accepted and spent the next five years teaching animal breeding classes and advancing the swine crossbreeding project. He added purebred Spots to the mix, trying various crosses, back-crosses, and tracking of crossbred sire data on about 400 litters/year.

Johnson’s five years of crossbreeding research had not gone unnoticed by his mentor, who assumed

*(Continued on next page)*



responsibilities as the animal science department head at the University of Nebraska in 1978. Omtvedt recruited his former student to fill the swine breeding position vacated by P.J. Cunningham. Johnson joined UNL with a 50:50 research and teaching appointment.

## Maternal Line Focus

Anxious to do something different on the research side, Johnson teamed up with well-known swine reproduction specialist Dwane Zimmerman. “There really wasn’t a lot of information about the genetics of reproduction, and Dwane was a good person to team up with to do a physiology/genetics research program,” he says.

“I’ve always thought that if you’re in research, you should try to do something that is forward-looking,” Johnson explains. By design and necessity, he has since tackled two of the pork industry’s greatest challenges — improving the reproductive efficiency of the sow herd and attempting to understand the heritability of disease resistance in pigs.

Genetic research is a slow process requiring patience and perseverance, both attributes Johnson has mastered over the years. “It takes a while in a selection experiment to understand the nature of the response. If you just look at one generation at a time, you get lots of variation. So you have to stay with an objective for a while before you can evaluate it.”

“Litter size components of reproduction weren’t all that well understood and the heritability estimates for litter size were pretty low, indicating that there may not be much opportunity to select for (improved) reproduction in the pig,” he says. “But I wasn’t convinced that you couldn’t make progress.”

Litter size is a reflection of three components — ovulation rate, embryonic survival, and uterine capacity. Master those pieces of the puzzle and you can probably design a selection index to improve litter size.

In his early work, one experiment focused on direct selection for litter size, another on decreasing age at puberty, and a third on selecting for ovulation rate.

“Direct selection for ovulation rate didn’t change litter size greatly, so I was trying to figure out why. We had females that ovulated more eggs, but litter size was not improving, so it had to have something to do with uterine capacity,” he speculated.

From 1979 to 1981, Johnson focused on establishing a genetic line that could be utilized through a multigenerational selection experiment. In 1981, the herd was permanently closed to outside genetics and the population was randomly split into two groups — a “control” group (no selection for litter size) and a “select” or index line that would undergo prescreening and intensive selection for litter size. A combination of ovulation rate and embryo survival served as the selection index criteria for what was to become the Nebraska Index Line (NIL).

Johnson reasoned that if he could measure litter size during gestation, he could simultaneously select for ovulation rate and uterine capacity. He turned to laparotomy, a procedure that requires a small incision near the navel of a bred gilt at 50 days of gestation. The reproductive tract is brought outside of the body cavity so researchers can palpate the uterus and count the number of corpora lutea on the ovaries for ovulation sites. The reproductive tract is carefully replaced and females are allowed to farrow naturally.

After five generations of selection, it was clear that ovulation rate was heritable because ovulation rates and number of fetuses at 50 days of gestation were increasing. But litter size lagged. “Something was happening after 50 days,” Johnson observes.

“Back then, physiologists thought most of the embryonic and fetal death loss occurred between 30 and 50 days of gestation. That may be the case in low-ovulating populations, but it clearly wasn’t the case in our select line. When ovulation rate exceeds uterine capacity by a fair bit, then you start to get losses in late gestation,” Johnson explains.

Complicating the theory of uterine crowding is the difficulty in measuring uterine size. “Measuring a uterus in a nonpregnant female is not a very good predictor of what happens during pregnancy. And, if you measure the uterus during pregnancy, it’s largely a function of how many fetuses are present,” he says.

The selection index was modified to place more emphasis on embryo survival, and by the 10th generation, ovulation rate had climbed from 13.5 in the control line (no selection) to 20 in the select line.

Litter size in the select line averaged about three more pigs per litter (12.5-13.0 vs. 9.5-10). Johnson felt he was on the right track, so the laparotomy procedure was abandoned in the 11th generation. In the 12th generation, he shifted more selection emphasis to number of pigs born alive. “Litter size just took off. And when it did, number of stillborns and mummified pigs declined,” he relates.

Johnson points out that the heritability of litter size in the select line was twice the popular estimate of 10%. “I think it is because ovulation rate is not a limiting variable. Females in the select line all had high ovulation rates, so what we were measuring was the heritability of uterine capacity.”

Selection for increased litter size continues, with the 30th generation born this spring. He shifted for a time to a two-stage selection program — selecting for ovulation rate, and then for large litter size among those with high ovulation rates. “The response went up sharply, especially in a line derived from the control line that had lower ovulation rate. Selection in recent generations has been for live pigs per litter, and numbers continue to go up,” Johnson reports.

The knock on the Nebraska Index Line, reported in the National Pork Producers Council (NPPC) Maternal Line



Program (see: [http://nationalhogfarmer.com/mag/farming\\_genetic\\_evaluation\\_maternal/index.html](http://nationalhogfarmer.com/mag/farming_genetic_evaluation_maternal/index.html)) in 2000, was that the pigs were born too small, grew too slow, and had too much backfat. Johnson acknowledges those concerns, but reminds that the line was closed in 1981 and no selection pressure was placed on postweaning performance.

In the last 10 years, the single reproductive trait in the selection index is live-born pigs/litter, plus he has added selection for increased growth rate and lower backfat levels. “Of course, they’re not close to the industry’s pigs yet, but the select lines now average about 25 lb heavier at 180 days with about 0.25 inch less backfat than the control line. And those lines have been a really valuable resource for molecular genetics work that tries to find genes that control reproduction,” he adds.

## Sow Longevity Study

A natural extension of Johnson’s genetics of reproduction work is a gilt development and sow longevity study. “We are looking at different gilt development options, controlling how fast they grow; how much backfat they have, using nutritional manipulation; then tracking their reproductive performance through four parities,” he explains. (See [http://nationalhogfarmer.com/genetics-reproduction/sow-gilt/farming\\_effects\\_energy\\_intake/](http://nationalhogfarmer.com/genetics-reproduction/sow-gilt/farming_effects_energy_intake/)). The study is nearly two-thirds complete.

Although he hasn’t pinpointed the most common reasons sows fall out of the breeding herd, he has learned some things that are not related. For example, some argue that gilts must weigh at least 300 lb and have a minimum backfat level. In his study, restricted feed intake held gilts to 220-230 lb at breeding, but some have gone on to produce 60 pigs in four parities, he explains. Conversely, some gilts mated at 340 lb never had a litter. “I think there is a positive association between those things, but perhaps not nearly as strong as some people believe,” he adds.

“Early puberty is probably the variable that is most highly correlated with lifetime productivity,” Johnson declares. He believes producers, and some previous researchers, begin boar exposure too late — 170-180 days of age. “They aren’t measuring puberty; they are measuring response to when they start boar exposure.”

In the Nebraska trials, gilts are first exposed to boars at 140 days of age. Both boars and gilts are moved to a central area separate from where they were raised. Boars are exposed to groups of 10-15 gilts for about 15 minutes, every day. “When they hit puberty, those gilts lock right up,” Johnson says. “I’d like to see our nucleus breeders record age at puberty, but it will be hard to get them to do that because it’s a costly and labor-intensive trait to measure.”

## Disease Resistance

Reinforcing his commitment to forward-looking research, about 10 years ago Johnson began looking at the

genetic implications of the two biggest profit robbers in the pork industry — porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus.

“There seems to be genetic variation for just about everything, so I thought there probably is genetic variation for response to PRRS virus,” he explains.

A trial was set up to challenge PRRS-negative pigs to a specific strain of the virus. “Pigs really responded differently. Every pig became viremic (replicating the virus), but within four days, some pigs had just a little spike in their temperature, a little blip in their growth, then cleared the virus and started to gain again. Others got sick and stayed sick for 2-3 weeks. Some never recovered,” he reports. “The nature of that response differed between two distinct, genetically different populations. That’s evidence that there is underlying genetic variation.”

“Although I’m sure you could be effective in selecting for a PRRS-resistant population, you wouldn’t have many pigs to sell,” he points out.

That’s where genomics comes in. A national project is underway to identify the genetic markers associated with resistance to PRRS. The goal would be to map the gene frequencies of the pigs that got really sick and those that didn’t. “It’s a really good application of genomic technology, but funding is hard to come by,” he says.

## Circovirus is Tougher

Circovirus is doubly difficult to work with in a genetic research environment because it exists everywhere and is hard to grow in a laboratory setting.

The percentage of nonvaccinated pigs that show phenotypic signs of circovirus in the Nebraska herd is about 15%, but it’s just 4-5% in some herds.

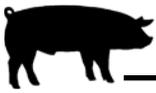
“So we’re vaccinating everything to protect a fairly small percentage of susceptible pigs. The (circovirus) vaccines are good, and they work well, but at \$1.50/pig, it’s costing over \$150 million to protect a pretty small number of pigs. Strictly from an economic standpoint, if we had resistance to circovirus, it would save pork producers a lot of money,” he says.

## Handling “the Pig Part”

Funding and complexity of disease resistance work remains an obstacle. Infecting pigs with a virus destroys their salvage value; the phenotypic data and viremia profile is important and expensive to collect; necropsy is costly, but essential; and there’s the added cost of genomics. “But the motivation for this research is that we’d like to produce pigs with fewer pharmaceuticals,” he notes.

In the waning years of his career, Johnson feels his role is to design the experiments, and collect the phenotypic data and tissues — handling the pig part — and leave good data for the molecular geneticists to explore.

*(Continued on next page)*



## A Gratifying Change

One of the most satisfying changes Johnson has seen in his swine genetics career is the broad adoption of artificial insemination.

“In the ‘70s, we were trying to convince pork producers that crossbreeding had value, heterosis is important, and specific crosses had merit. Today, no one even questions that. Thirty-five years ago, no one would think about using a boar without ever seeing him. Now, they just call up their supplier, order the semen they need, inseminate the sows and know it will work; they’re happy,” he reflects.

## Industry Challenges

Johnson cites environmental and welfare-type regulations as the biggest challenges pork producers face going forward. Initially, he felt those driving these initiatives were simply meddling.

“It sort of made me mad, but when I stopped and thought about it as a consumer, I, too, want to know how products are made and want to feel good about what I am buying. Why would we think that the consumers of pork should be any different?” he asks.

The challenge is that the general public no longer can drive past a farm and see the pigs. “That creates a bit of a black-box effect, so they are concerned about how their food is being raised,” he observes. “People who buy pork want to know that it is a safe product and it has been raised humanely.”

Another ongoing challenge Johnson sees is producers’ ability to handle new technology. “I’m sure they will be faced with sexed boar semen, as well as the possibility of delivering pharmaceuticals in their feeds, such as through modified corn hybrids that stimulate the immune system.

“These are some of the issues I think pork producers will face, and they will have to evaluate the cost-benefit ratio of that technology, which means they will have to be well educated,” Johnson notes.

## Reflections

Johnson is grateful for the work ethic instilled in him by his parents. He lists Joe Whiteman, Oklahoma State sheep geneticist, and Irv Omtvedt as the two most influential teachers and mentors.

He’s most proud of the Irvin and Wanda Omtvedt Professor of Animal Science appointment he received in 2003, a position he currently holds. Also high on his list of awards is an OSU Outstanding Alumni award in 1999, the Darrell W. Nelson Excellence in Graduate Student Advising award in 2005, and being named an American Society of Animal Sciences fellow in research in 2008.

The best advice he can give students interested in the swine industry is to do an internship in a modern pork production system. “Learn as much as you can about the industry; understand the biology of pig production, connect with producers, get involved,” he says.

Johnson is a big fan of internship programs. “Many students have a vision of pig production that is outdated — smelly, dirty, high labor. The modern, updated units are clean and well run, and the animals are well managed. That’s why the internship experience is so important. They get a different feeling from the quality of that work experience.”

Johnson has traveled to over 20 countries, many states, and has consulted for numerous breeding stock firms. Looking back over his career, he says: “I don’t ever regret having gone to pigs. I still enjoy cattle, but from a genetic standpoint, there are more traits that are important in pigs, they are a litter-bearing animal, and their generation interval is shorter. In cattle, it takes 15 years to do what we do in 3-4 years in pigs. I feel good about my career in pig genetics,” he adds.

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# Effects of Distillers Dried Grains With Solubles (DDGS) and Energy Restriction During Development on Growth and Puberty of Gilts

Age at puberty did not differ between gilts developed with ad libitum access to a 20% DDGS or corn grain diet, but pubertal development is delayed in gilts developed with 25% energy restriction.

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## Summary

*This experiment evaluated the effects of three gilt developmental regimens on growth and pubertal development. All gilts were allowed ad libitum access to a corn grain diet from weaning to 123 days of age. Thereafter, they were developed from 123 to 235 days of age with 1) ad libitum access to a corn grain diet, 2) ad libitum access to a diet with 20% distillers dried grains with solubles (DDGS), or 3) daily allotment of the 20% DDGS diet fed so that daily energy intake was 80% of that of ad libitum-fed gilts. Other nutrients were adjusted so they were not limiting. Daily feed intake for gilts with ad libitum access to the 20% DDGS diet was 95% ( $P < 0.01$ ) of that of their littermates consuming the corn grain diet, and thus, they gained 7.7 kg less from 123 to 235 days of age. They also had less longissimus muscle area at 235 days of age ( $2.5 \text{ cm}^2$ ,  $P < 0.01$ ), but did not differ in backfat thickness. Age at puberty did not differ between gilts developed with ad libitum access to the corn grain and DDGS diets. Daily feed intake for gilts on the restricted intake regimen was based on that of their littermates with ad libitum access to feed. Because of lower feed intake than expected in*

*the period around 140 days of age for gilts with ad libitum access to feed, the realized energy restriction for gilts on the restricted regimen was 75%, rather than the intended 80%. This caused body weight, backfat, and longissimus muscle area at 235 days of age to be substantially less than for their littermates with ad libitum access to feed ( $-22.5 \text{ kg BW}$ ,  $-1.15 \text{ cm BF}$ , and  $-5.3 \text{ cm}^2 \text{ LMA}$ ). Gilts developed with the energy restriction regimen were 10.9 days older ( $P < 0.01$ ) at puberty than their unrestricted littermates.*

## Introduction

In previous research supported by the Nebraska Pork Producers Association, our group evaluated the effects of restricting feed intake in developing gilts on reproductive performance through four parities. Biological and economic analyses of the data are summarized in the 2010 *Nebraska Swine Report*. The research reported here is an extension of that study. Thus, a brief overview of the findings will aid in understanding the objectives and design of the experiment reported herein.

A total of 661 gilts of two genetic lines were allowed either ad libitum access to a corn-soybean meal diet from weaning to breeding age of 235 days, or were developed so that daily energy intake from 123 days to 235 days was 75% of that consumed by ad libitum-fed gilts. The amounts of vitamins and minerals in the diet for restricted-fed gilts were increased so that these nutrients were not limiting. Fewer gilts developed with 75% energy restriction

expressed a pubertal estrus during the development period (86% vs. 96%), and those that did were older (177.5 vs. 174.1 days). Thereafter, differences in reproductive performance did not differ significantly between the groups, even though subsequent production through four parities per gilt entering the breeding herd was greater (25.6 vs. 23.8 total live pigs, 113.8 vs. 105.4 kg litter weaning weight) for those developed with restricted energy intake. Gilts of both lines responded similarly to the energy restriction regimen, but more gilts from the cross of the Nebraska selection line with an industry maternal line expressed pubertal estrus and were younger at puberty than gilts of an industry Large White-Landrace cross; they also had larger litters and greater lifetime production.

On the basis of those results, we have made lifetime sow productivity a major focus of the University of Nebraska-Lincoln (UNL) swine research group. Nutrition during development, genetics, and management of breeding females affect reproduction rate and lifetime production. Our goal is to develop management and genetic strategies that do not negatively affect early development, and improve subsequent sow lifetime productivity. Corn grain was the common energy source in the diet when our initial experiment was conducted. Since then, the ethanol industry has greatly expanded and DDGS is commonly used in swine diets. Producers have expressed concerns that puberty may be delayed when gilts are fed diets containing significant amounts of DDGS.

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Our initial work indicated that restricting energy intake in developing gilts is detrimental to their early sexual development but may enhance subsequent lifetime productivity. In retrospect, the 25% restriction of energy that we used may have been too severe. Thus, the next step in our long-term project was to compare the effects of allowing developing gilts ad libitum access to either a corn grain diet or a diet containing 20% DDGS to those given a diet containing 20% DDGS, but fed to restrict energy intake to 80% of that consumed by ad libitum-fed gilts. This report contains the developmental data through 235 days of age for 206 gilts developed with these regimens.

## Materials and Methods

### Dietary Regimens and Gilt Management

Project gilts were born in two batches (Replications, Rep) during July 2009 (Rep 1, n = 93) and November 2009 (Rep 2, n = 113). All gilts were the L45X used in our previous research. They were from litters produced by second and third parity Nebraska selection Line 45 sows that had been inseminated with semen of Danbred NA maternal line boars. Project gilts were from a total of 48 litters, 24 per replication. After weaning, gilts were managed alike in the nursery until approximately 60 days of age when they were moved to the growing-finishing facility where they were assigned to pens by age. Littermates were placed in different pens, in groups of 9 to 11 per pen. There were nine pens in Rep 1, and 12 in Rep 2. When gilts were assigned to pens, pens within age groups were randomly assigned to one of three treatments, described below, so that littermates were on different treatments. Treatments were initiated when gilts reached 123 days of age. From 60 to 123 days of age, all gilts were allowed ad libitum access to a corn-soybean meal-based

**Table 1. Composition of experimental diets.**

Ingredient, %	Corn, ad libitum		DDGS, ad libitum		DDGS, restricted	
	CA	DGSA	DGSA	DGSR	DGSA	DGSR
Corn	76.98	60.23	60.23	53.22	60.23	53.22
Soybean meal	17.00	13.75	13.75	20.00	13.75	20.00
DDGS	0.00	20.00	20.00	20.00	20.00	20.00
Tallow	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.48	1.48	1.48	2.05	1.48	2.05
Limestone	0.65	0.65	0.65	0.73	0.65	0.73
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix <sup>b</sup>	0.25	0.25	0.25	0.32	0.25	0.32
Trace mineral premix <sup>c</sup>	0.15	0.15	0.15	0.00	0.15	0.00
Trace mineral premix <sup>d</sup>	0.00	0.00	0.00	0.19	0.00	0.19
Calculated composition						
Crude protein, %	14.21	16.98	16.98	19.27	14.21	19.27
Lysine, %	0.69	0.72	0.72	0.88	0.69	0.88
Calcium, %	0.64	0.64	0.64	0.81	0.64	0.81
Phosphorus, %	0.61	0.66	0.66	0.79	0.61	0.79
ME <sup>e</sup> , Mcal <sup>f</sup> /kg	3.45	3.54	3.54	3.51	3.45	3.51

<sup>a</sup>DDGS = dried distillers grains with solubles.

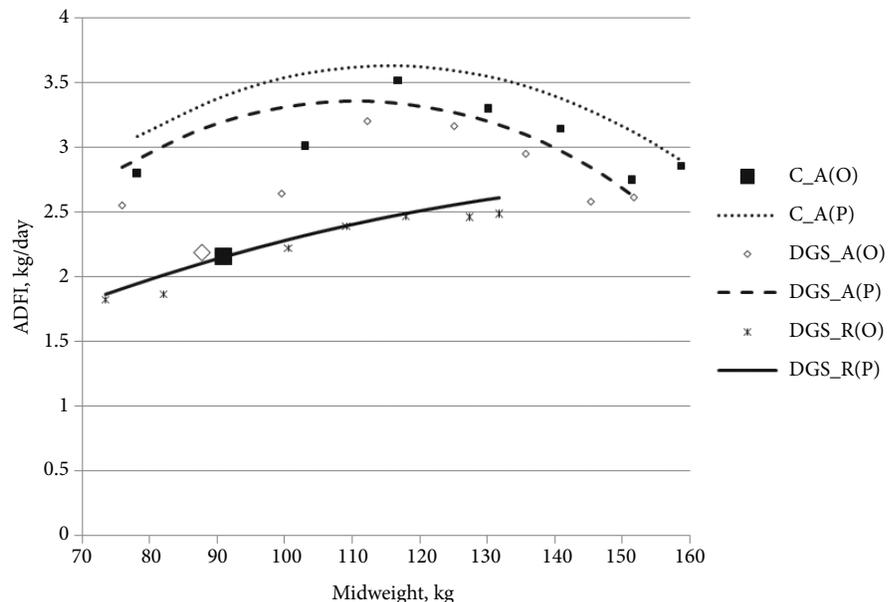
<sup>b</sup>Supplied per kilogram of diet at 0.25% inclusion: vitamin A as retinyl acetate, 5,500 IU; cholecalciferol, 550 IU;  $\alpha$ -tocopherol acetate, 30 IU; menadione sodium bisulfite, 4.4 mg; riboflavin, 11 mg; d-pantothenic acid, 22 mg; niacin, 33 mg; vitamin B<sub>12</sub>, 33 mg.

<sup>c</sup>Supplied per kilogram of diet at 0.15% inclusion: Zn (as ZnSO<sub>4</sub>), 128 mg; Fe (as FeSO<sub>4</sub>•H<sub>2</sub>O), 128 mg; Mn (as MnO), 30 mg; Cu (CuSO<sub>4</sub>•5H<sub>2</sub>O), 10.5 mg; I (as Ca(IO<sub>3</sub>)•H<sub>2</sub>O), 0.26; Se (as Na<sub>2</sub>SeO<sub>3</sub>), 0.26 mg.

<sup>d</sup>Supplied per kilogram of diet at 0.1875% inclusion: Zn (as ZnSO<sub>4</sub>), 159 mg; Fe (as FeSO<sub>4</sub>•H<sub>2</sub>O), 159 mg; Mn (as MnO), 38 mg; Cu (CuSO<sub>4</sub>•5H<sub>2</sub>O), 13 mg; I (as Ca(IO<sub>3</sub>)•H<sub>2</sub>O), 0.32; Se (as Na<sub>2</sub>SeO<sub>3</sub>), 0.3 mg.

<sup>e</sup>ME = Metabolizable energy.

<sup>f</sup>Mcal = Megacalorie.



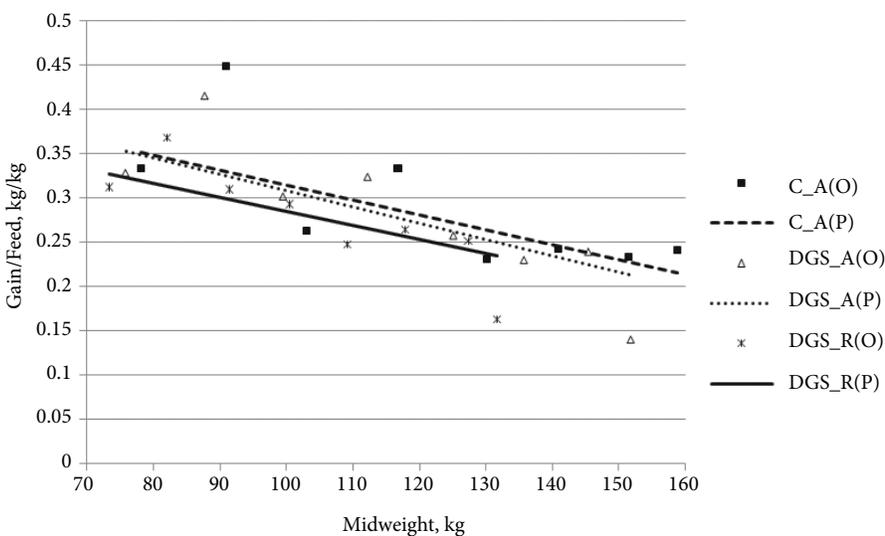
**Figure 1. Average daily feed intake plotted against midweight for each period for gilts developed with a corn grain diet fed ad libitum (CA), 20% DDGS diet fed ad libitum (DGSA), and DDGS diet fed at 75% of ad libitum (DGSR): markers (O) are observed mean intake, lines (P) are predicted means from regression ( $CA > DGSA$  and  $DGSR < \frac{1}{2}(CA + DGSA)$ ,  $P < 0.01$ ).**



**Table 2.** Mean weight (BW), backfat (BF), and longissimus muscle area (LMA) at 123 (D<sub>123</sub>) and 235 (D<sub>235</sub>) days of age, change in traits from 123 to 235 days of age, and age at puberty (AP) for gilts developed with corn diet fed ad libitum (CA), DDGS diet fed ad libitum (DGSA), and DDGS diet fed at 80% (DGSR)<sup>1</sup>.

Treatment	BW, kg		BF, cm		LMA, cm <sup>2</sup>		Gain from 123 to 235 d			
	Day <sub>123</sub>	Day <sub>235</sub>	Day <sub>123</sub>	Day <sub>235</sub>	Day <sub>123</sub>	Day <sub>235</sub>	BW, kg	BF, cm	LMA, cm <sup>2</sup>	AP
CA	71.9	163.7	1.20	3.30	23.9	47.4	91.7	2.10	23.5	164.0
DGSA	70.2	154.3	1.17	3.06	24.5	44.9	84.1	1.89	20.4	166.4
DGSR	69.7	136.5	1.10	2.03	24.0	40.9	66.7	0.93	16.9	176.1
Contrasts among means										
C vs DDGS	1.7	9.4**	0.03	0.24	-0.6	2.5**	7.7**	0.20	3.1**	-2.5
R vs A	-1.3	-22.5**	-0.09	-1.15**	-0.2	-5.3**	-21.2**	-1.06**	-5.1**	10.9**

<sup>1</sup>\*\* $P < 0.05$ , all other contrasts,  $P > 0.1$ .



**Figure 2.** Average efficiency, gain/feed, plotted against midweight for each period for gilts developed with a corn grain diet fed ad libitum (CA), 20% DDGS diet fed ad libitum (DGSA), and DDGS diet fed at 75% of ad libitum (DGSR): markers (O) are observed mean intake, lines (P) are predicted means from regression (G:F did not differ among treatments,  $P > 0.30$ ).

diet, and were managed alike. A three-phase growing-finishing diet was used: phase 1, 1.15% lysine (60 days to 36.3 kg); phase 2, 1.0% lysine (36.3 to 59 kg); and phase 3, 0.90% lysine (59 kg to 123 days).

The experimental treatments were three different gilt developmental regimens. These were 1) CA, ad libitum access to a corn-soybean meal diet from 123 to 235 days of age; 2) DGSA, ad libitum access to a corn-soybean meal diet that contained 20% DDGS; and 3) DGSR, a corn-soybean meal diet containing 20% DDGS fed daily at 80% of the amount consumed by

gilts on diets CA and DGSA. Diets are described in Table 1. The CA and DGSA diets were formulated to contain 0.70% lysine, 0.70% Ca, and 0.60% P. Calculated proportions of these nutrients were slightly greater than those amounts.

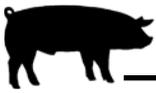
Gilts receiving the DGSR regimen were fed so that energy intake was approximately 80% that of gilts on the other diets, but proportions of protein, vitamins, and minerals, except selenium, in the diet were increased, so intake of these nutrients per unit of body weight was expected to be the same as for gilts developed with ad libitum access to feed.

Energy restriction was achieved by predicting intake with a quadratic equation of average daily feed intake on body weight of ad libitum-fed gilts. Gilts were weighed at 123 days of age and every 14 days thereafter until they were 235 days of age. Feed intake was recorded for each pen receiving CA and DGSA regimens each 14-day period. Regression of feed intake on midweight during the period was used to establish the relationship between daily feed intake and body weight. The predicted ad libitum intake (based on the projected body weight for the upcoming two-week period) was multiplied by 0.80 to determine the daily feed intake for gilts on the DGSR regimen. The DGSR diet contained 0.88% lysine, 0.81% Ca, and 0.79% P.

All pens were approximately 2.15 x 4.3 m, providing a minimum of 0.84 m<sup>2</sup> per pig. Pen floors were one-third solid and two-thirds slatted. The allotment of feed for pens on the DGSR regimen was placed on the solid portion in two meals per day at approximately 8 a.m. and 3 p.m.

Backfat thickness (BF) and longissimus muscle area (LMA) at the 10<sup>th</sup> rib were recorded at 123 days of age when treatments were initiated, and every 28 days thereafter until gilts were 235 days of age. When the oldest gilt in each pen was 140 days of age, gilts were moved by pen to an adjacent room where boar exposure and estrus detection occurred. The date of first observed estrus and each additional

*(Continued on next page)*



estrus were recorded. After the last measurements were recorded at 235 days of age, gilts were moved to the breeding barn.

### Traits and Data Analysis

Average daily feed intake (ADFI) and the ratio of gain to feed intake (G:F) for each 14-day period were calculated for each pen and analyzed on a pen basis. Body weight (BW), BF, LMA, and age at puberty were analyzed using records of each gilt. Models for ADFI and G:F included replication and treatment. Models for BW, BF, and LMA included replication, treatment, and the random effects of pen and litter of gilt. Repeated measures models accounting for the correlation structure among repeated measures over time (pens for ADFI and G:F, and gilts for other traits) were used. Mean ages of gilts at the beginning and end of the experiment (124.5 and 235.1 days, respectively) were very close to target ages of 123 and 235 days, but variation among pens within replication existed. To account for that variation, the age deviation of each gilt at each period from the target age (123, 137, ..., 235 days) was included as a covariate in models of BW, BF, and LMA, fitted within treatment, to adjust means to the specified target ages from 123 to 235 days of age. The means for CA and DGSA were compared to determine whether responses differed between gilts allowed ad libitum access to diets with and without DDGS. The mean for DGSR was compared to the average means of CA and DGSA to estimate the effect of an 80% energy restriction.

### Results

A curvilinear pattern of feed intake with increasing weight occurred for gilts allowed ad libitum access to feed (Figure 1). Average daily intake increased until weight was approximately 120 kg, and declined thereafter. At the same weight, gilts consuming DDGS (regimen DGSA) ate 95.3% ( $P < 0.01$ ) as much feed per kg body weight per day as gilts fed the corn

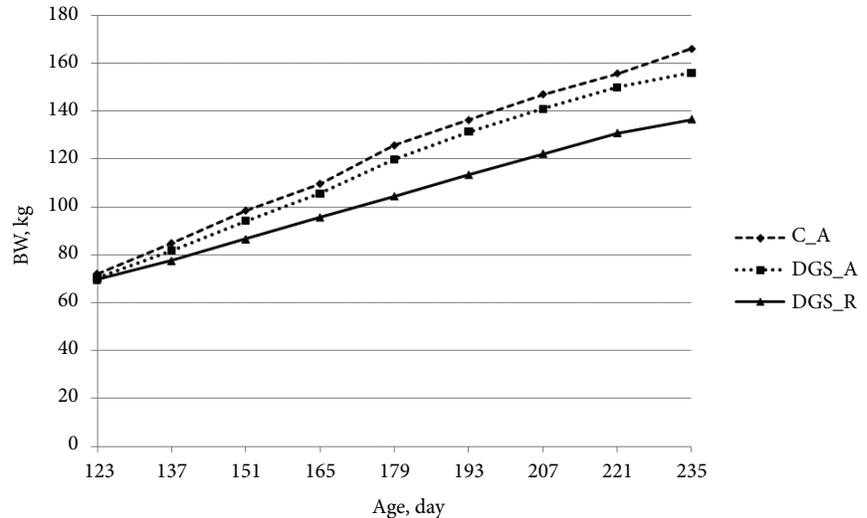


Figure 3. Average body weight at each age from 123 to 235 days for gilts developed with corn-based diet fed ad libitum (CA), 20% DDGS diet fed ad libitum (DGSA), and DDGS diet fed at 80% of ad libitum (DGSR).

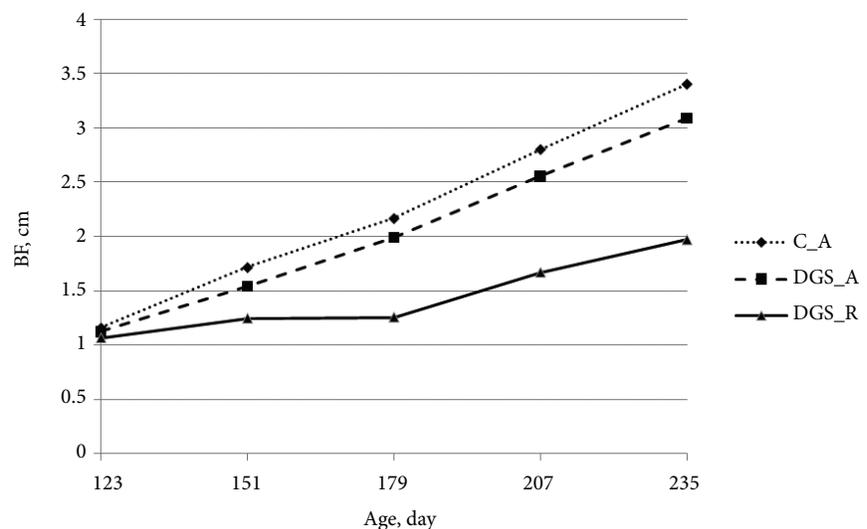


Figure 4. Average backfat thickness at ages from 123 to 235 days for gilts developed with corn based diet fed ad libitum (CA), 20% DDGS diet fed ad libitum (DGSA), and DDGS diet fed at 80% of ad libitum (DGSR).

grain diet (CA regimen), and that difference was consistent across all periods, ranging from 94.4 to 96.1%.

The experimental objective was to restrict gilts on the DGSR regimen to 80% of the daily feed intake per kg of body weight as gilts allowed ad libitum access to feed. However, the actual restriction was somewhat greater than intended. On average, during the entire feeding period, ADFI per kg of body weight for gilts on the DGSR regimen consumed 75.3% as

much feed as those allowed ad libitum access to feed. The actual restriction was greater during the first five 14-day feeding periods, ranging from 70.0 to 74.3%. During the last three periods, these gilts consumed 77.5, 82.2, and 85% of feed daily as their littermates on the DGSA regimen ate at these same weights.

Feed intake for the restricted regimen was based on feed intake for gilts allowed ad libitum access to feed. In both replications, feed intake

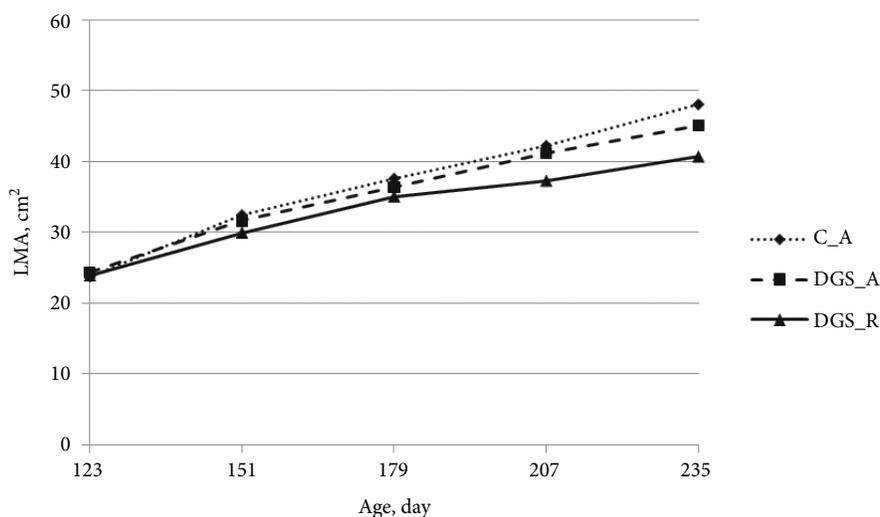


Figure 5. Average longissimus muscle area at ages from 123 to 235 days for gilts developed with corn based diet fed ad libitum (CA), 20% DDGS diet fed ad libitum (DGS\_A), and DDGS diet fed at 80% of ad libitum (DGS\_R).

in Period 2 for gilts on the CA and DGS\_A regimens was markedly less than in Period 1 and Period 3. The ADFI in Period 2 for gilts on the ad libitum treatments was 1.96 and 2.31 kg in Reps 1 and 2 respectively. The average of these amounts is illustrated with the enlarged markers in Figure 1. The reduction in Rep 1 was noticed immediately and occurred because a batch of corn was suspected to contain mycotoxins. The corn was replaced, and feed intake immediately increased. The presence of mycotoxins in the corn was subsequently confirmed with laboratory analyses. No explanation exists for the lower ADFI in Period 2 of Rep 2. Because ADFI for gilts on the DGS\_R regimen was based on ADFI of their littermates on the other regimens, and data for both Reps was used in the regression analyses, their feed allotment for that period and subsequent periods was affected.

The G:F ratio did not differ among treatments (Figure 2), although it was somewhat less for gilts on the DGS\_R regimen than for littermates with ad libitum access to feed. Consistent with results of previous experiments, the G:F declined linearly as weight increased.

Differences in BW at 235 days of age and in BW gain from 123 to 235 days of age are consistent with differences in ADFI. When allowed ad libitum access to feed, gilts consuming a diet with 20% DDGS had reduced ADFI (Figure 1) and, thus, gained less weight and weighed less at 235 days of age than gilts consuming the corn grain diet (Table 1). Backfat deposition over the gilt developmental period did not differ greatly between gilts consuming corn grain and those consuming DDGS, but the increase in LMA from 123 to 235 days of age was less for gilts consuming a diet with DDGS, and thus they had less LMA

at 235 days, than those consuming a diet with only corn grain. As expected, gilts on the DGS\_R regimen gained less weight, deposited less BF, and had less LMA at 235 days than their littermates with ad libitum access to feed. The development of body weight, BF, and LMA with age is illustrated in Figures 3, 4, and 5, respectively.

The gilt developmental regimen did not affect the proportion of gilts that expressed their pubertal estrus during the developmental period. Of the 206 gilts that started the experiment at 123 days of age, 204 survived through 235 days of age and completed the developmental period. A pubertal estrus was recorded for 201 of those gilts. Thus, whether gilts expressed a pubertal estrus during the developmental period was not affected by the developmental regimen. However, their age at puberty was (Table 2). Age at puberty did not differ ( $P > 0.10$ ) between gilts consuming corn grain or DDGS diets when allowed ad libitum access to feed. However, those on the DGS\_R regimen were 10.9 days older ( $P < 0.05$ ) at puberty than their littermates with ad libitum access to feed. This result is consistent with the previous experiment in which restricting daily energy intake using a corn grain diet also caused gilts to be older at puberty.

<sup>1</sup>Rodger K. Johnson and Phillip S. Miller are professors, and Justin W. Bundy is a research technologist in the UNL Animal Science Department; Matthew W. Anderson is manager, and Jeffery M. Perkins, Arlan A. Kettlehake, Donald R. McClure, and Thomas E. McGargill are research technicians at the UNL Swine Research Farm.



# Effects of Distillers Dried Grains With Solubles (DDGS) and Ractopamine on Swine Growth and Carcass Value

Ractopamine fed for 25 days before market in diets with and without DDGS increased gain to feed ratio by 17.9% and total carcass value by 2.6%.

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**Roman Moreno**  
**Matthew W. Anderson**  
**Thomas E. McGargill**  
**Donald R. McClure**  
**Jeffrey M. Perkins**  
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**Phillip S. Miller**  
**Rodger K. Johnson<sup>1</sup>**

## Summary

*The effects of including the beta agonist ractopamine (RAC, Paylean®) in diets with and without distillers dried grains with solubles (DDGS) were assessed with 240 pigs during a 117-day feeding experiment. Pigs were moved to the grower facility when they were approximately 60 days of age and placed in 24 pens, each with five barrows and five gilts. Those in 12 pens were fed a corn grain (CG) diet without DDGS; the others were fed a diet comprised of 20% DDGS. From day 92 to 117, RAC at the rate of 4.5 ppm was included in the diet fed to half of the pigs on each diet. Barrows and gilts responded similarly to DDGS and to RAC when included in both CG and DDGS diets. Pigs receiving the DDGS diet consumed 0.087 kg less feed per day during the first 92 days on feed ( $P < 0.01$ ), and thus weighed 2.27 kg less at day 92 ( $P < 0.10$ ). Because they weighed less, they also had less backfat and longissimus muscle area ( $P < 0.10$ ). Diet did not affect G:F. Thereafter, there were no differences in performance between pigs fed CG and DDGS diets. During the last 25 days, pigs consuming RAC*

*ate 0.220 kg less feed per day, gained 1.98 kg more total weight, had 0.22 cm less backfat, and 2.15 cm<sup>2</sup> greater longissimus muscle area than those eating diets without RAC ( $P < 0.05$ ). As a result, they had 0.043 kg/kg greater G:F. Pigs consuming the CG diet had greater hot carcass weight than those fed DDGS, but there was no difference in carcass value per kg of carcass weight. Pigs fed RAC had heavier (1.74 kg) and leaner (0.97%) carcasses than those fed diets without RAC ( $P < 0.01$ ), resulting in carcasses with \$3.52 greater total value ( $P < 0.05$ ) and \$0.012 greater value per kg carcass weight.*

## Introduction

The swine research literature contains many reports of the effects of feeding diets that contain up to 40% DDGS. Observed responses vary among experiments, but in most cases, diets with 30% DDGS have not negatively affected performance. It is also well established that feeding diets with the beta-agonist ractopamine (RAC, Paylean®) during the last 28 days of the finishing period increases lean growth rate if dietary amino acid concentrations are sufficient to meet the pig's needs for increased lean growth rate.

In a previous experiment conducted at the University of Nebraska–Lincoln (UNL) swine research unit and reported in the 2009 *Nebraska Swine Report*, increasing the amount of DDGS in the diet from 0 to 40% caused a linear decrease in average daily gain (ADG) during the early

growing period, but had no effect during the finishing period. In addition, the inclusion of RAC in the diet at 4.5 ppm during the last 28 days of the finishing period did not affect ADG, ADFI, G:F, or measures of carcass leanness, even though amounts of soybean meal and essential amino acids in the diet were increased. That experiment was conducted with 40 individually-fed pigs.

We conducted an experiment in which 240 pigs, five barrows and five gilts in each of 24 pens, were fed corn-soybean diets without DDGS or with 20% DDGS for 117 days. Paylean® was included in the diet of half of the pigs at 4.5 ppm during the last 25 days, the other half of the pigs continued on their respective diet without the inclusion of Paylean. Feed intake and growth performance were recorded during each phase, and carcass data for each pig were obtained when pigs were harvested. The objective of the experiment was to determine whether the effects on performance and carcass value of RAC in diets with and without DDGS differ.

## Methods

### *Animals and Facilities*

A total of 240 terminal cross pigs were used. Their dams were a maternal line produced within the UNL swine research herd that, over several generations, has been maintained by mating females in rotation with Danbred NA Large White, Danbred NA Landrace, and Nebraska Line 45



**Table 1. Ingredients and calculated composition of the dietary treatments, as-fed basis.**

Phase Treatment	Grower 1		Grower 2		Finisher 1		Finisher 2			
	Corn	DDGS	Corn	DDGS	Corn	DDGS	Corn	Corn+RAC	DDGS	DDGS+RAC
<b>Ingredient, %</b>										
Corn	71.275	60.425	74.475	62.375	80.185	67.825	86.565	72.230	74.975	62.365
Soybean meal	23.750	19.750	21.000	18.250	15.500	13.000	9.250	23.230	6.000	18.200
DDGS <sup>a</sup>	0.000	15.000	0.000	15.000	0.000	15.000	0.000	0.000	15.000	15.000
Tallow	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Dicalcium phosphate	1.200	0.950	0.850	0.600	0.700	0.500	0.600	0.550	0.350	0.300
Limestone	0.890	1.025	0.840	0.975	0.840	0.975	0.825	0.610	0.950	0.770
NaCl	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
Vitamin premix <sup>b</sup>	0.200	0.200	0.200	0.200	0.150	0.150	0.150	0.150	0.150	0.150
Trace mineral premix <sup>c</sup>	0.150	0.150	0.150	0.150	0.100	0.100	0.100	0.100	0.100	0.100
L-lysine•HCl	0.150	0.190	0.150	0.150	0.150	0.150	0.150	0.460	0.175	0.540
DL-methionine	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.095
L-tryptophan	0.015	0.010	0.000	0.000	0.000	0.000	0.010	0.045	0.000	0.045
L-threonine	0.050	0.000	0.035	0.000	0.075	0.000	0.050	0.150	0.000	0.110
Paylean <sup>®</sup>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.025
<b>Calculated composition</b>										
Crude protein, %	17.05	18.53	16.02	17.95	14.00	15.99	11.64	17.31	13.39	18.45
Lysine, %	0.99	1.00	0.92	0.93	0.78	0.79	0.61	1.22	0.63	1.24
Calcium, %	0.69	0.67	0.58	0.57	0.53	0.53	0.49	0.44	0.47	0.43
Phosphorus, %	0.59	0.57	0.51	0.50	0.46	0.46	0.42	0.46	0.41	0.45
ME <sup>d</sup> , Mcal <sup>e</sup> /kg	3.40	3.48	3.42	3.49	3.43	3.50	3.43	3.41	3.51	3.49

<sup>a</sup>DDGS = dried distillers grains with solubles.

<sup>b</sup>Supplied per kilogram of diet at 0.2% inclusion: vitamin A as retinyl acetate, 4,400 IU; cholecalciferol, 440 IU;  $\alpha$ -tocopherol 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<sub>12</sub>, 26.4 mg.

<sup>c</sup>Supplied per kilogram of diet at 0.15% inclusion: Zn (as ZnSO<sub>4</sub>), 128 mg; Fe (as FeSO<sub>4</sub>•H<sub>2</sub>O), 128 mg; Mn (as MnO), 30 mg; Cu (CuSO<sub>4</sub>•5 H<sub>2</sub>O), 10.5 mg; I (as Ca(IO<sub>3</sub>)•H<sub>2</sub>O), 0.26 mg; Se (as Na<sub>2</sub>SeO<sub>3</sub>), 0.26 mg.

<sup>d</sup>ME = Metabolizable energy

<sup>e</sup>Mcal = megacalorie

boars. Semen from Danbred LW and LR lines is used consecutively for two generations, and each third generation females are mated with Nebraska Line 45 boars. Genetic composition of dams was approximately 6/7 Danbred maternal lines and 1/7 Nebraska Line 45. These rotation cross females were inseminated with semen of Danbred NA terminal line boars to produce the experimental pigs.

Litters were born during April 2009. Experimental pigs were selected from the nursery at approximately 60 days of age and moved to a curtain-sided finishing building on June 1. Controls with thermostats regulated the height of the curtains to control ventilation. Pigs were assigned randomly to pens within sex so that each pen contained five barrows and five gilts. Each pen was 1.52 m x 4.88 m, providing 0.74 m<sup>2</sup> space per pig with 1/3 solid and 2/3 slatted flooring. Pens contained self feeders and nipple waterers. Pens of pigs were randomly

assigned to one of four treatments.

#### Dietary Treatments

During the first 92 days of the feeding period, pens of pigs received either a corn/soybean meal diet with 0% DDGS (denoted treatment CG) or a corn/soybean meal diet with 20% DDGS, denoted DDGS. During the last 25 days, 4.5 ppm RAC was added to the diet fed to one-half of the pens receiving CG and DDGS diets. Thus, six pens were assigned to each of the diet-RAC combinations. Diets used during each of the growing phases are described in Table 1. Amounts of protein and certain amino acids in the diet were increased in diets containing RAC.

#### Traits

Pigs were weighed on the day they were placed in pens and every 14 days thereafter. Feed intake for each 14-day

period and feeder weights were recorded every 14 days and used to calculate average daily feed intake (ADFI) and G:F for the 92-day and 25-day periods. Pigs were scanned for 10<sup>th</sup> rib backfat depth (BF) and longissimus muscle area (LMA) on day 92 and at the termination of the experiment on day 117. Pigs were shipped in two loads on day 118 and 119 to the Triumph Foods processing plant, St Joseph, Mo., where carcass data of each pig were collected.

Pen performance traits were ADFI, ADG, and G:F for the periods day 0 to 92 and day 92 to 117. Individual pig traits were body weight (BW), BF, and LMA at 92 and 117 days, and weight gain from day 0 to 92, and day 92 to 117. Hot carcass weight (HCW), BF depth, longissimus muscle depth (LD), percentage carcass lean (%L), total carcass value (CV\$), and carcass value per kg of HCW (CV\$/kg) were analyzed. Iodine values, a measure of softness of the fat, were measured in the jowl, belly, and loin of each

(Continued on next page)



carcass.

## Results

Of the 240 pigs placed on test, 236 completed it and had records for all traits. Three pigs were removed from their pens 14 days after initiation of the experiment because they were unthrifty. One pig died midway through the experiment. Each of these pigs was in a different pen.

Initial weights for pigs on the different treatments were nearly identical and did not affect subsequent BW (Tables 2 and 3). ADFI from day 0 to 92 was  $0.087 \pm 0.025$  kg greater ( $P < 0.01$ ) for pigs consuming the CG diet than for those consuming a diet containing 20% DDGS, which caused them to weigh  $2.27 \pm 1.24$  kg more ( $P < 0.10$ ) after 92 days on feed (Table 2). Differences in ADG between pigs consuming the CG and DDGS diets were relatively small, and pigs on both diets had nearly identical G:F. No differences in ADFI, ADG, or G:F between pigs consuming CG and DDGS diets during the last 25 days occurred.

Pigs consuming diets with RAC consumed  $0.220 \pm 0.050$  kg less feed per day than those eating diets without RAC, without a reduction in ADG. Thus, they converted feed to BW more efficiently ( $0.043 \pm 0.013$  kg/kg G:F,  $P < 0.01$ ). No interaction between diet and RAC occurred ( $P > 0.5$ ); the effects of RAC were similar when included in CG and DDGS diets.

During the first 92 days, pigs consuming the CG diet without DDGS gained  $2.05 \pm 1.17$  kg more BW and thus weighed  $2.27 \pm 1.24$  kg more at the end of the period (Table 3). They also had greater BF depth ( $0.09 \pm 0.06$  cm,  $P < 0.10$ ) and LMA ( $1.43 \pm 0.69$ ,  $P < 0.05$ ). BF and LMA were fitted in models with BW as a covariate to determine whether the observed increases were an underlying biological effect of CG vs. DDGS, or whether they were simply due to increased weight. After adjustment for BW, there were no differences between pigs fed CG and DDGS diets. Thus, the increased weight gain and greater BF

**Table 2. Pen mean growth traits<sup>1</sup> during Period 1 (92 days) for pigs fed corn grain (CG) or CG plus 20% dried distillers grain (DDGS) diets and Period 2 (25 days) when CG and DDGS diets without RAC (RAC<sup>-</sup>) and with RAC (RAC<sup>+</sup>) at 4.5 ppm were fed<sup>2</sup>.**

Item	BWB, kg	BWE, kg	ADFI, kg	ADG, kg	G:F, kg/kg
Period 1, 92 days					
CG	21.4	106.5	2.38	0.92	0.39
DDGS	21.1	104.2	2.30	0.90	0.39
CG - DDGS		$2.27 \pm 1.24^\dagger$	$0.087 \pm 0.025^{**}$	$0.021 \pm 0.012$	$-0.0056 \pm 0.0040$
Period 2, 25 days <sup>1</sup>					
CG	106.5	129.9	3.07	0.93	0.31
DDGS	104.2	127.5	3.00	0.91	0.31
CG - DDGS		$2.40 \pm 1.66$	$0.073 \pm 0.050$	$0.022 \pm 0.042$	$0 \pm 0.013$
RAC <sup>-</sup>	105.7	128.0	3.14	0.89	0.28
RAC <sup>+</sup>	105.0	129.3	2.92	0.95	0.33
RAC <sup>+</sup> - RAC <sup>-</sup>		$1.32 \pm 1.66$	$-0.220 \pm 0.050^{**}$	$0.062 \pm 0.042$	$0.043 \pm 0.013^{**}$

<sup>1</sup>BWB and BWE = body weight at the beginning and ending of the period, respectively, ADFI = average daily feed intake, ADG = average daily gain, and G:F = ratio of gain to feed intake.

<sup>2</sup>No interaction of diet by RAC treatment occurred for any trait ( $P > 0.5$ ).

$^\dagger$  =  $P < 0.10$ .

$^{**}$  =  $P < 0.01$ .

**Table 3. Period 1 (92 days) beginning (BWB) and ending (BWE) mean weights, BW gain, and ending backfat thickness (BF) and longissimus muscle area (LMA) for barrows (B) and gilts (G) fed corn grain (CG) or CG plus 20% dried distillers grain (DDGS) diets<sup>1</sup>.**

Item	BWB, kg	BW Gain, kg	BWE, kg	BF, cm	LMA, cm <sup>2</sup>
CG	21.4	85.1	106.5	1.92	33.9
DDGS	21.1	83.1	104.2	1.83	32.5
B	21.5	87.5	109.0	2.01	33.4
G	21.1	80.7	101.7	1.74	33.1
CG - DDGS		$2.05 \pm 1.17^\dagger$	$2.27 \pm 1.24^\dagger$	$0.09 \pm 0.06^\dagger$	$1.43 \pm 0.69^*$
B - G		$6.8 \pm 1.17^{**}$	$7.24 \pm 1.83^{**}$	$0.27 \pm 0.06^{**}$	$0.28 \pm 0.69$

Sex x diet interaction did not exist for any trait ( $P > 0.1$ ).

$^\dagger$  =  $P < 0.10$ .

$^{**}$  =  $P < 0.01$ .

and LMA for pigs fed the CG diet were due entirely to their greater feed intake (Table 2). Barrows and gilts responded similarly to DDGS as there was no interaction of sex with diet ( $P > 0.10$ ).

Changes in BW, BF, and LMA during the last 25 days of the feeding period were nearly identical for pigs consuming CG and DDGS diets (Table 4). All the differences that existed on day 117 were due to those that existed at day 92. No interactions of diet with RAC, sex with diet, or sex with RAC occurred for any trait (all  $P > 0.3$ ); barrows and gilts receiving CG and DDGS diets responded similarly to RAC. RAC-fed pigs had greater BW gain ( $1.98 \pm 0.97$ ,  $P < 0.01$ ) during the

last 25 days of feeding and  $0.22 \pm 0.07$  cm less BF and  $2.15 \pm 0.71$  cm<sup>2</sup> LMA on day 117 than pigs that consumed diets without RAC. As expected, barrows grew faster and had greater BF during the first 92-day period (Table 3) and the last 25-day period (Table 4).

When harvested, pigs that were fed the CG diet had  $1.97 \pm 0.98$  kg greater HCW, but they differed very little in BF, LD, or percentage lean (Table 5). Based on the payment grid in place at that time, pigs fed the CG diet had somewhat greater total carcass value, due to heavier weights, but less value per kg of HCW, which caused a small increase in the number discounted for too great HCW.



**Table 4. Mean weight at the beginning (BWB) and ending (BWE) of Period 2 (25 days) for barrows (B) and gilts (G) fed corn grain (CG) or 80% CG:20% dried distillers grain (DDGS) diets without (RAC<sup>-</sup>) and with Ractopamine (RAC<sup>+</sup>) at 4.5 ppm<sup>1</sup>.**

Item	BWB, kg	BW gain, kg	BWE, kg	BF, cm	LMA, cm <sup>2</sup>
CG	106.5	23.4	129.9	2.20	40.6
DDGS	104.2	23.2	127.5	2.18	39.4
NP	105.7	22.3	128.0	2.30	38.9
P	105.0	24.3	129.3	2.08	41.1
B	109.0	24.0	133.0	2.30	40.2
G	101.7	22.6	124.46	2.09	39.8
CG - DDGS		0.14 ± 0.97	2.40 ± 1.66	0.03 ± 0.07	1.24 ± 0.71†
RAC <sup>+</sup> - RAC <sup>-</sup>		1.98 ± 0.97**	1.32 ± 1.66	-0.22 ± 0.07**	2.15 ± 0.71**
B - G		1.33 ± 0.97**	8.59 ± 1.66**	0.21 ± 0.07**	0.40 ± 0.71

<sup>1</sup>Interaction of diet x RAC, sex x diet, and sex x RAC did not occur for any trait ( $P > 0.10$ ).

\*\* =  $P < 0.01$ .

**Table 5. Mean hot carcass weight (HCW), backfat (BF) and longissimus muscle depth (LD), total carcass value (CV) and carcass value per kg for barrows (B) and gilts (G) fed corn grain (CG) or 80% CG:20% dried distillers grain (DDGS) diets for 92 days and DG and DDGS diets without (NP) and with Paylean (P) at 4.5 ppm for 25 days<sup>1</sup>.**

Item	HCW	Lean%	BF	LD	CV, \$	CV/kg, \$
CG	99.1	51.5	23.2	62.4	136.94	1.38
DDGS	97.2	51.5	23.5	62.1	135.26	1.39
NP	97.3	51.0	24.4	61.2	134.34	1.38
P	99.0	52.0	22.4	63.3	137.86	1.39
B	101.6	51.0	24.6	62.8	140.03	1.37
G	94.7	52.0	22.1	61.7	132.18	1.39
CG - DDGS	1.97±0.98*	0.00±0.3	-0.32±0.61	0.36 ± 0.91	1.68 ± 1.43	-0.009±.006
RAC <sup>+</sup> - RAC <sup>-</sup>	1.74±.98†	0.97±.3**	-0.97±.61**	2.12±.91	*3.52±1.43	*0.012±.006†
B - G	6.93±.98**	-0.96±.3**	2.49±.61	1.05±.91	7.85±1.43**	-0.017±.006**

<sup>1</sup>Interaction of diet x RAC, sex x diet, and sex x RAC did not occur for any trait ( $P > 0.05$ ).

† =  $P < 0.10$ .

\* =  $P < 0.05$ .

\*\* =  $P < 0.01$ .

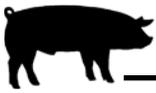
Pigs that consumed the diet with RAC during the last 25 days had 1.74 ± 0.98 kg greater HCW ( $P < 0.10$ ) and carcasses with less BF (-0.97 ± .61 mm,  $P < 0.01$ ) and 2.12 ± .91 mm greater LD ( $P < 0.05$ ). Thus, their lean percentage was 0.97 ± 0.3 % greater ( $P < 0.01$ ), their total carcass value was \$3.52 ± 1.43 greater ( $P < 0.05$ ), and their carcass value per kg HCW was \$0.012 ± .063 greater ( $P < 0.10$ ) than that of pigs that consumed diets without RAC.

Carcass cuts from pigs that consumed the DDGS diet had greater iodine values in the jowl, belly, and loin ( $P < 0.01$ ). Mean belly iodine values were 62.4 and 68.3 for pigs fed diets with and without DDGS, respectively. Pigs that consumed diets with RAC also had greater iodine values in belly and jowl ( $P < 0.05$ ), but not in the loin ( $P > 0.50$ ). Belly iodine values were 64.9 and 65.8 for pigs fed diets without and with RAC. Greater

iodine values occur when concentration of unsaturated fatty acids in the fat occurs. With that increase, fat tissue becomes softer. The result here is consistent with most other reports. Feeding diets with DDGS increases the concentration of unsaturated fatty acids and causes softer fat, particularly in the belly. In addition, feeding diets with RAC may also slightly increase concentration of unsaturated fatty acids in the belly. Interaction of diet by RAC did not occur. Therefore, effects of DDGS and RAC on belly softness are additive. The net effect is that an increased proportion of carcasses with soft bellies is expected when pigs are fed diets with DDGS and RAC than in those fed diets without these ingredients.

The carcass value data reported here are for the market and the Triumph Foods grid that existed in September 2009. Pigs with these same performance traits will have different value today and will differ among packer grids. Differences in value among pigs fed CG and DDGS or those receiving diets with and without RAC will be more consistent over time and markets than actual values. Similarly, costs of diets formulated as those described in Table 1 will vary across time. Producers can use current ingredient prices and the growth, efficiency, and carcass value data presented herein to make decisions about the economic value of feeding CG and DDGS diets with and without RAC.

<sup>1</sup>T. Kellner is an undergraduate animal science student; Roman Moreno and Justin W. Bundy are research technologists in the UNL Animal Science Department; Matthew W. Anderson is manager of the UNL Swine Research Farm; Thomas E. McGargill, Donald R. McClure, Jeffrey M. Perkins, and A. Kettlehut are research technicians at the UNL Swine Research Farm; Phillip S. Miller and Rodger K. Johnson are professors in the UNL Animal Science Department.



# Effects of Incorporation of a Yeast-dried Milk Product in Creep Feeding and Phase-1 Nursery Diets on Growth Performance and Circulating Immunoglobulin A of Pigs

Creep feeding during the time period of days 7 to 21 postfarrowing increases litter nursing performance. Inclusion of a yeast-dried milk product improves the creep-feeding response.

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## Summary

Two feeding experiments were conducted to evaluate the effects of incorporating of a yeast-dried milk product in creep feeding and Phase-1 nursery diets. In Experiment 1, 24 sows and their litters were assigned to pens based on anticipated farrowing date. Dietary treatments included: 1) no creep, 2) control creep (CTL), and 3) experimental creep (10% yeast-dried milk) and were randomly allotted to pens (eight litters/treatment). Creep diets (1.50% true ileal digestible Lys) were fed ad libitum from day 7 after birth until weaning (day 21) in a pan creep feeder. Pigs fed experimental and CTL creep diets tended to have greater (14.70 and 14.56 vs. 13.38 lb;  $P = 0.10$ ) weaning BW compared to pigs not receiving creep feed. Overall, pigs fed experimental creep had 40% greater ( $P = 0.02$ ) ADFI compared to pigs fed the CTL, and tended to have 10.7% greater ( $P = 0.11$ ) ADG compared to noncreep fed pigs. In Experiment 2, a total of 108 weaned pigs were selected based on the mean BW of pigs from each of the three treatments in Experiment 1 and randomly allotted to one of 18 pens (six pigs/pen, six pens/treatment). Creep diets from Experiment 1 were continually fed dur-

ing Phase 1 (day 0 to 7) followed by a common diet during Phase 2 (day 7 to 21) and Phase 3 (day 21 to 28). Thus, pigs that received creep diets during the nursing period received the same diet during Phase 1. Pigs fed experimental creep had greater BW compared to CTL and noncreep fed pigs ( $P < 0.05$ ) during week 1 to 3. Overall (day 0 to 28), pigs fed experimental creep had greater (1.16 vs. 0.99 and 0.93 lb;  $P = 0.03$ ) ADG and ADFI (1.61 vs. 1.42 and 1.30 lb;  $P = 0.002$ ) compared to the CTL and noncreep fed pigs. At weaning, pigs fed CTL creep had greater ( $P = 0.03$ ) immunoglobulin A compared to noncreep fed pigs; however, there were no differences among pigs fed experimental creep and other treatments. At the end of Phase 1 (day 7), greater ( $P = 0.03$ ) circulating immunoglobulin A was observed in pigs fed experimental creep compared to noncreep fed pigs.

## Introduction

It may take several days for weaning pigs to adapt to starter feed due to the change from sow's milk to solid feed, and the stresses induced by the weaning process. During this transition period, pigs may have increased disease susceptibility and lose weight. Therefore, reducing the required time after weaning for pigs to start eating may prevent weaning-associated problems and stimulate growth performance. A previous experiment conducted at the University of Nebraska demonstrated that a yeast-dried milk

product can be incorporated in Phase 1 nursery diets and maintain acceptable growth performance. Therefore, two experiments were conducted to: 1) determine the effects of incorporating a yeast-dried milk product in creep feed (day 7 to 21 post-farrowing) diets; and 2) evaluate effects of creep feeding on pig growth performance and circulating immunoglobulin (Ig) A in the subsequent Phase 1 nursery period.

## Materials and Methods

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Nebraska–Lincoln (UNL).

### *Animals, Experimental Design, and Dietary Treatments*

**Experiment 1.** Twenty-four sows and their litters were selected and assigned to pens in two rooms (12 sows/room) based on their anticipated farrowing day. Cross-fostering was conducted in order to have 10 to 11 pigs/litter. Treatments were randomly assigned to pens. Creep feeding was initiated on day 7 postfarrowing and continued until weaning (day 21). Each litter was monitored twice a day and creep feeders were refilled as needed. Litters were weighed and creep feed disappearance was estimated on day 7, 14, and 21 postfarrowing. Blood samples were collected on day 21 from all piglets. Fecal and blood samples



**Table 1. Diet ingredients and nutrient composition (% as-fed basis).**

Experiment Ingredients	Experiment 1 and Phase-1 of Experiment 2			Experiment 2
	CTL creep	Experimental		Phase 3
		creep	Phase 2	
Corn	40.56	33.22	49.93	53.22
Soybean meal, 46.5% CP	6.00	6.00	20.50	20.50
Spray-dried porcine plasma	6.00	6.00	3.00	3.00
Select Menhaden fish meal	6.00	6.00	7.50	7.50
DairyLac 80 <sup>1</sup>	25.00	22.82	12.50	9.38
Extruded soy protein concentrate	10.00	10.00	0.00	0.00
Dicalcium phosphate, 18.5% P	0.40	0.00	0.60	0.50
Limestone	0.48	0.63	0.25	0.30
Salt	0.30	0.30	0.30	0.30
Zinc oxide	0.30	0.30	0.30	0.30
Vitamin premix <sup>2</sup>	0.25	0.25	0.25	0.25
Trace mineral premix <sup>3</sup>	0.15	0.15	0.15	0.15
Lysine•HCl, 78% lysine	0.31	0.04	0.38	0.34
DL-Methionine	0.17	0.19	0.18	0.14
L-Threonine	0.08	0.10	0.17	0.13
Mecadox 2.5	1.00	1.00	1.00	1.00
Corn Oil	3.00	3.00	3.00	3.00
Yeast-dried milk <sup>4</sup>	0.00	10.00	0.00	0.00
Total	100.00	100.00	100.00	100.00
<b>Calculated composition</b>				
CP, %	22.34	24.67	21.82	21.91
Total Lys, %	1.65	1.65	1.58	1.56
tid <sup>5</sup> Lys, %	1.50	1.50	1.47	1.42
Ca, %	0.85	0.85	0.83	0.81
P, %	0.74	0.73	0.74	0.71
aP, %	0.52	0.52	0.49	0.45
ME <sup>6</sup> , kcal/lb	1,536	1,567	1,531	1,533
Lactose, %	20.00	20.00	10.00	7.50

<sup>1</sup>DairyLac 80 is a sweet and dried whey soluble product (International Ingredient Corporation, St. Louis, MO) containing 3.2% CP and 0.06% Lys (analyzed composition) and 80% lactose.

<sup>2</sup>Vitamin premix supplied per kg of diet: vitamin A (as retinyl acetate), 5,500 IU; vitamin D (as cholecalciferol), 550 IU; vitamin E (as -tocopheryl acetate), 30 IU; vitamin K (as menadione dimethylpyrimidinol bisulfate), 4.4 mg; riboflavin, 11.0 mg; d-pantothenic acid, 22.05 mg; niacin, 33.0 mg; vitamin B<sub>12</sub> (as cyanocobalamin), 33.0 mg.

<sup>3</sup>Trace mineral premix containing: copper (as CuSO<sub>4</sub>•5H<sub>2</sub>O), 10 mg/kg; iodine (as Ca (IO<sub>3</sub>)•H<sub>2</sub>O), 0.25 mg/kg; Iron (FeSO<sub>4</sub>•2H<sub>2</sub>O), 125 mg/kg; manganese (MnO), 15 mg/kg; Selenium (Na<sub>2</sub>SeO<sub>3</sub>), 0.3 mg/kg; Zinc (ZnSO<sub>4</sub>•H<sub>2</sub>O), 125 mg/kg.

<sup>4</sup>Yeast-dried milk is a mixture of 50% dried near dated-milk and 50% dried brewer's yeast containing 17.4% lactose, 33% CP and 1.82% Lys (analyzed composition).

<sup>5</sup>tid: true ileal digestible

<sup>6</sup>ME: metabolizable energy



**Figure 1. Pan creep feeder used in Experiment 1.**

from selected pigs were taken at weaning prior to allocation to the nursery study (Experiment 2).

Diet composition is shown in Table 1. In both experiments, all diets were fed in meal form and formulated to meet or exceed requirements identified by NRC (1998) for nursery pigs. Yeast-dried milk product used in the experimental diets was produced from 50% dried brewer's yeast and 50% dried near-dated milk (International Ingredient Corp., St. Louis, Mo.). This product contained 32.5% CP, 17.5% lactose, and 2.33% Lys (analyzed composition). Dietary treatments included: 1) no creep, 2) control creep (CTL), and 3) experimental creep (10% yeast-dried milk). Creep diets (1.50% true ileal digestible Lys) were provided from day 7 after birth until weaning (day 21) in a pan creep feeder (see Figure 1).

**Experiment 2.** A total of 108 weaned pigs (54 barrows and 54 gilts) were selected based on the mean BW of pigs from each of the three treatments in Experiment 1, and randomly allotted to 1 of 18 pens (6 pigs/pen, 6 pens/treatment). Therefore, any differences (variation) of creep feeding effects observed from Experiment 1 were maintained in the group of pigs allotted to Experiment 2. All pigs were housed in a temperature-controlled room with one water nipple, one self-feeder, and continuous lighting. Pigs were fed ad libitum in a three-phase feeding period including: Phase 1, day 0 to 7; Phase 2, day 7 to 21; and Phase 3, day 21 to 28. Dietary treatments from Experiment 1 were maintained in Phase 1 of Experiment 2. The no-creep fed pigs in Experiment 1 were fed the control creep in Phase 1 of Experiment 2. All pigs were fed a common diet in Phase 2 and Phase 3. Pigs were weighed weekly and blood samples were collected via jugular venipuncture at day 0 (weaning) and day 7 prior to weighing. Serum was harvested following centrifugation (20 min at 1,500 × g) and frozen (-20°C) for subsequent analyses.

*(Continued on next page)*



A porcine specific, enzyme-linked immunosorbent assay (ELISA) was used to quantify circulating immunoglobulin (Ig) A (Bethyl Laboratories, Inc.; Montgomery, Tex.). Serum was diluted at 1:1,000 with assay buffer for analysis of IgA.

*Statistical Analysis*

All data were analyzed by MIXED procedure (SAS Inst. Inc., Cary, N.C.). Experiment 1 was randomized completely block design. In this experiment, each litter was considered an experimental unit and a random effect. Room (block) is a fixed effect. There was no room effect in this experiment; therefore, room × treatment interaction was not included in the model. Birth weight was used as a covariate to compare the difference among treatments on weaning BW, ADG, and ADFI. In Experiment 2, each pen was an experimental unit and a random effect. Weaning BW was used as a covariate to compare the difference among treatments on BW. In both experiments, the model included dietary treatment as a fixed effect. All means are presented as least-squares means (± SEM). The pdiff option of SAS was used to compare the differences among treatments.

**Results and Discussion**

*Growth Performance*

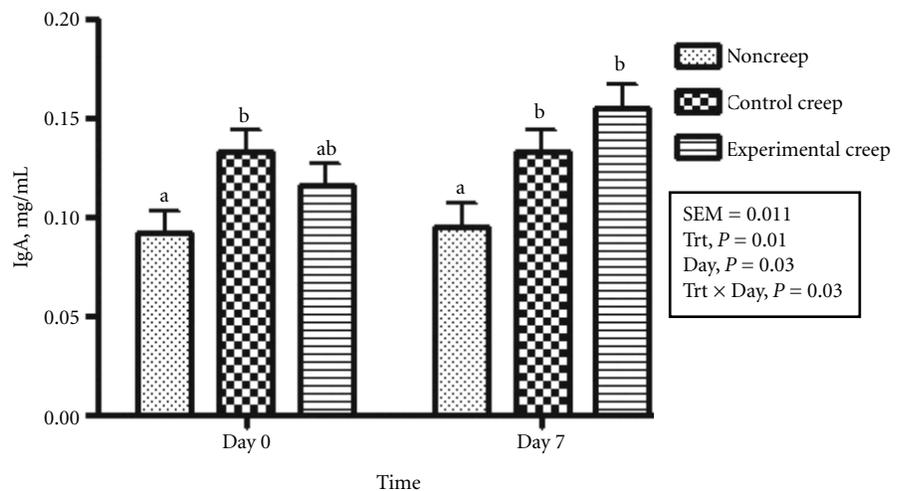
**Experiment 1.** The preweaning growth performance is provided in Table 2. Using birth weight as a covariate, pigs fed experimental or CTL creep feed tended ( $P = 0.10$ ) to show increased weaning BW (14.70 and 14.56 vs. 13.38 lb) compared to pigs not receiving creep feed. There were no differences among treatments on ADG in Phase 1 (day 7 to 14); however, greater ( $P = 0.03$ ) ADG in pigs receiving experimental or CTL creep (0.64 and 0.63 vs. 0.53 lb) compared to pigs not receiving creep diet in Phase 2 (day 14 to 21) was observed. Compared to the CTL creep, experimental

**Table 2.** Effects of creep feeding on growth performance of preweaning pigs (Experiment 1).

	Dietary treatments			SEM	P-values (main effect)
	No creep	CTL creep	Experimental creep		
Number of litters	8	8	8		
Litter size	11.4	11	10.9	0.5	0.79
BW, lb					
Birth wt	2.86	3.30	3.39	0.18	0.10
Wean wt <sup>1</sup>	13.38 <sup>a</sup>	14.56 <sup>b</sup>	14.70 <sup>b</sup>	0.42	0.10
Week 1 (day 7 to 14)					
ADG, lb	0.59	0.61	0.60	0.03	0.88
ADFI, lb	0.00	0.03	0.04	0.00	0.05
Week 2 (day 14 to 21)					
ADG, lb	0.53 <sup>a</sup>	0.63 <sup>b</sup>	0.64 <sup>b</sup>	0.03	0.03
ADFI, lb	0.00	0.03	0.05	0.00	0.05
Week 1, 2 (day 7 to 21)					
ADG, lb	0.56	0.62	0.62	0.02	0.11
ADFI, lb	0.00	0.03	0.05	0.00	0.02

<sup>1</sup>Birth weight is used as a covariate to calculate and compare weaning wt, ADG, and ADFI among the treatments.

<sup>a-b</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).



**Figure 2.** Effect of creep feeding on circulating concentration of Immunoglobulin A in weaning pigs (Experiment 2). Each bar represents the least-square mean (± SEM) of 36 observations. Bars with different superscript differ ( $P < 0.05$ ).

creep did not have additional effects on ADG in the preweaning period; however, weaning BW was numerically greater in pigs fed experimental creep diet. Overall (day 7 to 21), pigs fed experimental creep had greater (0.05 vs. 0.03 lb,  $P = 0.02$ ) ADFI compared to pigs fed the CTL creep and tended ( $P = 0.11$ ) to have greater ADG compared to pigs not receiving creep diets.

**Experiment 2.** The growth performance was presented in Table 3. At weaning (day 0), greater

( $P < 0.001$ ) BW was observed in pigs fed experimental or CTL creep compared to pigs not receiving creep feed in nursing period; however, there was a similar BW between the two creep treatments. The observed difference on BW at weaning resulted from the creep feeding during the nursing period (Experiment 1) and continued on to Experiment 2 where creep diets were continually fed through the end of Phase 1. A greater BW was observed in pigs fed experimental creep compared



**Table 3. Effects of creep feeding on growth performance of nursery pigs (Experiment 2)<sup>1</sup>**

Post-weaning	No creep	CTL creep	Experimental creep	SEM	P-value
Pen replications	6	6	6		
Number of pigs	36	36	36		
BW, lb					
day 0	14.50 <sup>a</sup>	16.26 <sup>b</sup>	16.70 <sup>b</sup>	0.20	< 0.001
day 7	18.33 <sup>a</sup>	18.81 <sup>a</sup>	20.11 <sup>b</sup>	0.33	0.004
day 14	24.16 <sup>a</sup>	24.62 <sup>a</sup>	26.73 <sup>b</sup>	0.66	0.03
day 21	33.75 <sup>a</sup>	33.51 <sup>a</sup>	36.04 <sup>b</sup>	0.73	0.03
day 28	44.42 <sup>ab</sup>	44.02 <sup>a</sup>	47.19 <sup>b</sup>	0.99	0.06
Phase 1 (day 0 to 7)					
ADG, lb	0.35 <sup>a</sup>	0.43 <sup>a</sup>	0.62 <sup>b</sup>	0.04	0.0008
ADFI, lb	0.35 <sup>a</sup>	0.43 <sup>a</sup>	0.58 <sup>b</sup>	0.03	0.0002
G:F, lb/lb	0.97	0.99	1.09	0.08	0.51
Phase 2					
day 7 to 14					
ADG, lb	0.79	0.84	0.97	0.05	0.07
ADFI, lb	1.02 <sup>a</sup>	1.11 <sup>a</sup>	1.34 <sup>b</sup>	0.06	0.005
G:F, lb/lb	0.78 <sup>a</sup>	0.76 <sup>ab</sup>	0.73 <sup>b</sup>	0.02	0.05
day 14 to 21					
ADG, lb	1.19 <sup>a</sup>	1.24 <sup>ab</sup>	1.40 <sup>b</sup>	0.06	0.05
ADFI, lb	1.72 <sup>a</sup>	1.80 <sup>ab</sup>	2.00 <sup>b</sup>	0.07	0.03
G:F, lb/lb	0.70	0.69	0.70	0.01	0.65
day 7 to 21					
ADG, lb	0.99 <sup>a</sup>	1.04 <sup>ab</sup>	1.18 <sup>b</sup>	0.05	0.04
ADFI, lb	1.37 <sup>a</sup>	1.46 <sup>a</sup>	1.67 <sup>b</sup>	0.06	0.01
G:F, lb/lb	0.74	0.72	0.71	0.01	0.18
Phase 3 (day 21 to 28)					
ADG, lb	1.37	1.46	1.65	0.10	0.17
ADFI, lb	2.13 <sup>a</sup>	2.35 <sup>b</sup>	2.53 <sup>b</sup>	0.07	0.005
G:F, lb/lb	0.64	0.61	0.65	0.03	0.69
Overall (day 0 to 28)					
ADG, lb	0.93 <sup>a</sup>	0.99 <sup>a</sup>	1.16 <sup>b</sup>	0.06	0.03
ADFI, lb	1.30 <sup>a</sup>	1.42 <sup>a</sup>	1.61 <sup>b</sup>	0.05	0.002
G:F, lb/lb	0.77	0.76	0.79	0.03	0.74

<sup>1</sup>No creep = pigs not fed creep diet in Experiment 1 but fed CTL creep diet in Phase 1 of Experiment 2; CTL creep = pigs fed CTL creep in Experiment 1 and Phase 1 of Experiment 2; Experimental creep = pigs fed experimental creep in Experiment 1 and Phase 1 of Experiment 2. Body weight at day 0 is used as covariate to calculate and compare BW among the treatments.

<sup>a-b</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).

to the CTL creep and noncreep fed pigs in Phase 1 (day 7;  $P = 0.004$ ), and Phase 2 (day 14 and 21;  $P = 0.03$ ). At the end of experiment, there was a tendency ( $P = 0.06$ ) to have greater BW in pigs fed experimental creep (47.19 lb) compared to the CTL creep (44.02 lb).

As expected, in comparison to CTL creep and noncreep fed pigs during the first week of the nursery period (day 0 to 7), pigs fed experimental creep had greater ADG (30 and 44%;  $P = 0.0008$ ) and ADFI (25 and 40%;  $P = 0.0002$ ). There was no differences in G:F ratio among the treatments. At the end of Phase 2 (day 21) when all pigs were fed a common diet, pigs fed experimental creep had greater (1.18 vs. 0.99 lb,  $P = 0.04$ ) ADG compared to

pigs not receiving creep feed; however, no difference between pigs fed experimental creep and the CTL diet were observed. In addition, a greater (1.67 vs. 1.46 and 1.37 lb;  $P = 0.01$ ) ADFI in pigs fed experimental creep compared to CTL creep and noncreep fed pigs, respectively, was observed in Phase 2. In Phase 3 (day 21 to 28), there were greater ( $P = 0.005$ ) ADFI in experimental creep and CTL creep fed pigs compared to noncreep fed pigs.

Overall (day 0 to 28), pigs fed experimental creep had greater ADG (14.7 and 19.8%;  $P = 0.03$ ) and ADFI (11.8 and 19.3%;  $P = 0.002$ ) compared to CTL creep and noncreep fed pigs. No differences on G:F among treatments were observed.

Our results indicate that consumption of creep feed increased ADG and ADFI in pigs compared to no creep feeding. In this study, feeding a creep diet containing yeast-dried milk and continuing the dietary regime for the first seven days in the nursery, resulted in a 3 lb greater BW at the end of the 28-day nursery period.

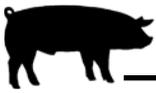
### Circulating Immunoglobulin

At weaning, pigs fed CTL creep had greater ( $P = 0.03$ ) IgA compared to noncreep fed pigs; however, there were no differences among pigs fed experimental creep and other treatments (Figure 2). At the end of Phase 1 (day 7), there were no differences on IgA between two groups of creep-fed pigs; however, pigs fed experimental creep significantly increased ( $P = 0.03$ ) circulating IgA compared to noncreep fed pigs. We predict that creep feeding may stimulate the mucosal immunity to mature earlier because of early exposure to feed antigens; therefore, it leads to a greater IgA titer in creep fed pigs.

### Conclusions

Within the farrowing and nursery systems used at the University of Nebraska, creep feeding was an effective method to increase ADFI and ADG of piglets during nursing and postweaning periods. The response appeared greater for pigs consuming creep diet containing a yeast-dried milk product. In addition, creep feeding may result in a change in circulating IgA of pigs; therefore, it may be beneficial to improve the health of nursery pigs. Future studies will focus on defining the mechanisms that mediate the responses in feed intake and growth performance associated with creep feeding.

<sup>1</sup>Huyen Tran and Erin E. Hinkle are graduate students; Justin W. Bundy is a research technologist; Thomas E. Burkey is an assistant professor and Phillip S. Miller is a professor in the UNL Animal Science Department.



# Evaluation of Soybean Meal with Genetically Modified Input Traits DP-356Ø43-5 Fed to Growing Pigs

The nutritional value of soybean meal derived from genetically modified soybeans containing event DP-356Ø43-5 is similar to the nontransgenic near-isoline control and commercially available soybean meal.

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## Summary

A growth experiment was conducted to evaluate the nutritional impact of soybean meal derived from genetically modified soybeans that expressed tolerance to glyphosate and acetolactate synthase-inhibiting herbicides by comparing data from growing pigs fed diets containing event DP-356Ø43-5 (356043) soybean meal and three other soybean meal sources. The treatments included diets containing soybean meal derived from: modified soybeans (356043), nontransgenic near-isoline soybeans (control), and two nontransgenic commercial soybean sources (reference). A total of 64 individually penned pigs (initial BW = 48.5 lb) were allotted to corn-soybean meal diets with the sole soybean meal content derived from one of the four treatment sources. Pigs were fed three dietary phases during the growth period to market weight (256 lb). Within each phase, data collected included body weights and feed disappearance in order to calculate ADG, ADFI, and G:F. At the conclusion of the feeding period, pigs were slaughtered and carcass characteristic data were collected. No differences were observed in the final weight (260 vs 260 lb), ADG (2.1 vs 2.1 lb), ADFI (5.6 vs 5.5 lb), or G:F (0.37 vs 0.38 lb/lb) between pigs fed diets containing the control and 356043, respectively. Also, no differences were observed in hot carcass weight (196 vs 196 lb), dressing

percentage (76.4 vs 76.7%), 10th-rib backfat (0.76 vs 0.70 in), and calculated carcass lean percent (52.2 vs 51.9%). These results suggest that the nutritional value of soybean meal derived from genetically modified soybeans containing event DP-356Ø43-5 is similar to the nontransgenic near-isoline control soybean meal.

## Introduction

It has become well known that the production of genetically modified crops continues to increase. Soybean production makes up a significant amount of genetically modified crops globally. Transgenic soybean line DP-356Ø43-5 was produced by inserting the *gat4601* and soybean acetolactate synthase (*gm-hra*) genes. The 356043 soybean plants expressed tolerance to the herbicidal active ingredient glyphosate and to acetolactate synthase (ALS)-inhibiting herbicides.

The objective of this experiment was to evaluate the potential nutritional impact of 356043 soybean meal by comparing growth data and carcass characteristics of swine fed diets containing 356043 soybean meal with those fed diets containing nontransgenic control soybean meal with comparable genetic background, or diets containing nontransgenic commercial soybean meal.

## Procedures

All procedures used in the experiment were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Nebraska–Lincoln (UNL). The study protocol was also reviewed and

approved by the Pioneer Animal Care and Use Committee.

## Animals, Housing, and Experimental Design

A total of 64 pigs (initial BW = 48.5 ± 0.9 lb) were randomly allotted to four dietary treatments based on weight and sex (barrows and gilts) in a randomized complete block design. Pigs were housed in an environmentally controlled building with two rooms and 32 individual pens per room.

## Diets and Animal Data

The four soybean sources used included: a nontransgenic near-isoline control; two nontransgenic commercial varieties (Pioneer brand varieties 92M72 and 93B15, Pioneer Hi-Bred Int. Inc., Johnston, Iowa); and a genetically modified variety (356043) with the insertion of the *gat4601* gene and the *gm-hra* gene. Event-specific, real-time PCR testing confirmed the presence of event DP-356Ø43-5 in 356043 soybean meal and its absence from control and reference soybean meals. Soybean meal samples were analyzed for DM, CP, EE, GE, CF, NDF, ADF, ash, Ca, P, and AA (Table 1). A three-phase feeding program was used for the experiment. Ingredient compositions of the diets are presented by phase in Table 2. All diets were formulated to meet or exceed estimated requirements for all nutrients (NRC, 1998). Samples of each diet were collected for nutrient analysis (DM, CP, EE, GE, CF, NDF, ADF, ash, Ca, P, and AA). Sub-samples were also used for PCR analysis to confirm the presence of the DP-356Ø43-5 event in the



**Table 1. Analyzed composition of the soybean meals used in experimental diets, as-fed basis.**

Item	Soybean source <sup>1</sup>			
	Control	356043	92M72	93B15
Moisture, %	6.20	4.60	5.50	4.80
Crude protein, %	48.90	50.90	49.90	48.10
Ether extract, %	1.20	2.20	2.00	1.30
GE <sup>2</sup> , Mcal <sup>3</sup> /lb	2.00	2.08	2.03	2.03
Crude fiber, %	4.00	2.80	3.00	4.50
NDF <sup>4</sup> , %	14.30	14.60	11.60	12.90
ADF <sup>5</sup> , %	6.50	5.50	4.20	6.00
Ash, %	6.20	6.10	6.50	6.10
Calcium, %	0.33	0.29	0.31	0.36
Phosphorus, %	0.75	0.80	0.79	0.70
<b>Indispensable AA<sup>6</sup>, %</b>				
Arginine	3.46	3.76	3.64	3.61
Histidine	1.31	1.42	1.32	1.30
Isoleucine	2.31	2.48	2.37	2.40
Leucine	3.89	4.20	3.96	3.90
Lysine	3.07	3.24	3.21	3.18
Methionine	0.67	0.71	0.73	0.70
Phenylalanine	2.47	2.66	2.48	2.49
Threonine	1.87	2.01	1.92	1.84
Tryptophan	0.72	0.74	0.70	0.68
Valine	2.46	2.66	2.50	2.51

<sup>1</sup>Soybean sources from which soybean meal was derived: Control = nontransgenic, near-isoline compared to 356043; 356043 = transgenic containing the event DP-356043-5; 92M72 and 93B15 = commercially available, nontransgenic varieties.

<sup>2</sup>GE = gross energy

<sup>3</sup>Mcal = megacalorie

<sup>4</sup>NDF = neutral detergent fiber

<sup>5</sup>ADF = acid detergent fiber

<sup>6</sup>AA = amino acids

**Table 2. Ingredient composition of experimental diets, as-fed basis.**

Ingredient, %	Soybean source <sup>1</sup>			
	Control	356043	92M72	93B15
<b>Phase 1: 55 to 130 lb</b>				
Nontransgenic corn	68.988	69.973	72.024	70.794
Soybean meal	28.394	27.036	24.989	26.318
L-Lysine 98%	0.000	0.000	0.022	0.000
Limestone	0.849	1.221	1.175	1.074
Dicalcium phosphate	0.769	0.770	0.790	0.814
Salt	0.500	0.500	0.500	0.500
Vitamin-mineral premix	0.500	0.500	0.500	0.500
<b>Phase 2: 130 to 200 lb</b>				
Nontransgenic corn	76.622	77.991	77.120	76.163
Soybean meal	20.867	19.451	20.349	21.365
DL-Methionine 99%	0.016	0.018	0.008	0.009
Limestone	0.946	0.986	0.979	0.899
Dicalcium phosphate	0.549	0.554	0.544	0.564
Salt	0.500	0.500	0.500	0.500
Vitamin-mineral premix	0.500	0.500	0.500	0.500
<b>Phase 3: 200 to 265 lb</b>				
Nontransgenic corn	80.136	80.942	82.152	81.394
Soybean meal	17.508	16.664	15.448	16.271
L-Lysine 98%	0.000	0.000	0.019	0.000
Limestone	0.960	0.999	0.972	0.911
Dicalcium phosphate	0.396	0.395	0.409	0.424
Salt	0.500	0.500	0.500	0.500
Vitamin-mineral premix	0.500	0.500	0.500	0.500

<sup>1</sup>Soybean sources from which soybean meal was derived: Control = nontransgenic, near-isoline compared to 356043; 356043 = transgenic containing the event DP-356043-5; 92M72 and 93B15 = commercially available, nontransgenic varieties.

356043 diets, and its absence from all control and reference diets.

Pigs were fed the respective diets in a three-phase feeding program (approximately: 55 to 130 lb, phase 1; 130 to 200 lb, phase 2; and 200 to 260 lb, phase 3). Feed and water were provided ad libitum throughout the experiment. Individual pig weights were recorded at the beginning of the experiment and at the conclusion of each of the three feeding phases. Daily feed allocations were recorded and the amount of feed remaining in the feeders was recorded each time the pigs were weighed. Pig data calculated included ADG, ADFI, and G:F.

#### Carcass Evaluation

Pigs were slaughtered over a period of three consecutive days at an average BW of 256 lb. The order that pigs were slaughtered was randomized among treatments within each harvest day. Live weights at slaughter and hot carcass weights were recorded. At approximately 24 hours postmortem dressing percent, 10th-rib fat thickness, LM area, and LM depth were recorded.

#### Statistical Analysis

Data were analyzed using a mixed model ANOVA (PROC MIXED, SAS Institute Inc., Cary, N.C.). Soybean meal, sex, and the soybean meal × sex interaction were fixed effects in the analysis of growth data and carcass data; room assignment was included as a random effect for growth data; room assignment and harvest day were included as random effects for carcass data. The individual pig was the experimental unit for all analyses. Estimate statements were used to generate the true comparison of interest in the experiment (control vs 356043) for each trait measured. The observed *P*-values generated from the estimate comparison statement determined whether the mean of the control group was statistically different from the mean of the 356043 group; differences between means were considered significant at *P* < 0.05. False discovery rate, as described by Benjamini and

(Continued on next page)



**Table 3. Analyzed nutrient composition of experimental diets, as-fed basis.**

Soybean source <sup>1</sup>	55 to 130 lb, phase 1				130 to 200 lb, phase 2				200 to 265 lb, phase 3			
	Control	356043	92M72	93B15	Control	356043	92M72	93B15	Control	356043	92M72	93B15
Item												
Moisture, %	9.40	9.50	9.00	9.10	11.80	11.60	11.50	11.60	11.70	11.70	11.40	11.30
Crude protein, %	18.20	17.90	18.10	18.60	15.00	15.20	15.30	14.80	13.90	13.40	13.50	13.70
Ether extract, %	2.90	2.90	3.10	3.20	3.20	3.40	3.10	3.20	3.00	3.20	3.20	3.20
GE <sup>2</sup> , Mcal <sup>3</sup> /lb	1.76	1.76	1.76	1.77	1.75	1.75	1.75	1.76	1.74	1.73	1.74	1.75
Crude fiber, %	2.50	2.40	2.30	2.20	1.80	1.60	2.00	1.70	2.60	2.20	2.20	2.10
NDF <sup>4</sup> , %	10.30	9.20	9.90	10.20	10.80	9.40	9.60	9.50	10.20	9.60	10.00	9.50
ADF <sup>5</sup> , %	5.00	4.10	4.70	4.00	4.70	4.40	4.50	4.50	4.10	3.90	3.80	3.30
Ash, %	4.50	4.60	4.60	4.80	3.90	4.20	4.10	3.70	4.00	3.80	4.10	3.90
Calcium, %	0.77	0.73	0.81	0.78	0.60	0.64	0.66	0.56	0.59	0.57	0.58	0.56
Phosphorus, %	0.52	0.50	0.53	0.52	0.45	0.46	0.45	0.44	0.40	0.40	0.39	0.40
<b>Indispensable AA<sup>6</sup>, %</b>												
Arginine	1.23	1.11	1.16	1.24	0.99	1.02	1.02	0.94	0.87	0.87	0.88	0.87
Histidine	0.57	0.53	0.54	0.58	0.43	0.43	0.43	0.41	0.39	0.37	0.37	0.38
Isoleucine	0.78	0.69	0.73	0.80	0.71	0.70	0.67	0.63	0.59	0.58	0.58	0.58
Leucine	1.65	1.55	1.58	1.69	1.47	1.49	1.45	1.44	1.38	1.34	1.35	1.36
Lysine	1.05	0.97	1.00	1.04	0.80	0.85	0.86	0.75	0.72	0.73	0.72	0.71
Methionine	0.28	0.27	0.27	0.27	0.27	0.27	0.26	0.25	0.22	0.23	0.22	0.22
Phenylalanine	0.94	0.85	0.88	0.96	0.79	0.78	0.77	0.77	0.70	0.68	0.68	0.69
Threonine	0.73	0.66	0.68	0.73	0.59	0.62	0.61	0.59	0.51	0.50	0.50	0.51
Tryptophan	0.26	0.23	0.25	0.25	0.20	0.20	0.20	0.20	0.19	0.18	0.17	0.18
Valine	0.88	0.79	0.82	0.91	0.80	0.79	0.78	0.74	0.69	0.67	0.67	0.67

<sup>1</sup>Soybean sources from which soybean meal was derived: Control = nontransgenic, near-isoline compared to 356043; 356043 = transgenic containing the event DP-356043-5; 92M72 and 93B15 = commercially available, nontransgenic varieties.

<sup>2</sup>GE = gross energy

<sup>3</sup>Mcal = megacalorie

<sup>4</sup>NDF = neutral detergent fiber

<sup>5</sup>ADF = acid detergent fiber

<sup>6</sup>AA = amino acids

Hochberg (1995), was applied across all traits analyzed to minimize the chance of falsely declaring a difference for a measured trait as significantly different when the difference may have only occurred by chance based on the number of measured traits. The false discovery rate adjusted *P*-value was reviewed if statistically significant differences generated from the estimate comparison statement were observed for a trait. Data from pigs fed diets containing the commercial soybean meals (92M72 and 93B15) were used in the estimation of experimental variability. Specific comparisons between the commercial reference with the control and 356043 groups were not generated from estimate statements. Instead, the reference group data were used to construct a 95% tolerance interval containing 99% of the observed growth and carcass trait values from pigs fed nontransgenic commercial soybean meal diets, as described by Graybill (1976). If there were significant differences, the false discovery rate adjustments were made, and data from the control and 356043 groups were then evaluated to determine whether or not the observed val-

ues were contained within the tolerance interval derived from the reference soybean meal groups. Tolerance intervals were generated by sex due to expected differences between the sexes.

### Results and Discussion

Nutrient concentrations within each phase were similar among the treatment groups (Tables 1-3). One mortality from the 356043 group occurred within the first 10 days after the start of the experiment. Necropsy determined that the mortality was a result of gastric ulceration.

Growth data, including weight, ADG, ADFI, and G:F, are presented in Table 4. All observed trait values were within the tolerance intervals calculated for each sex using the data from the commercial soybean meal treatment groups. There were differences observed ( $P < 0.05$ ) between the sexes for final BW, ADG, and ADFI. Barrows gained more weight and consumed more feed than gilts; however, no difference in feed efficiency between the sexes was observed. The data collected from the carcass characteristics are presented in

Table 5. Carcass differences ( $P < 0.05$ ) between sexes were as expected with barrows having heavier carcasses, more backfat, and greater loin depth than gilts.

There were no differences observed in the growth data between pigs fed diets containing the control soybean meal and pigs fed diets containing the 356043 soybean meal. All observed trait values were within the tolerance intervals calculated using the data from the commercial soybean meal treatment groups. Hot carcass weight, dressing percent, and 10th-rib backfat were similar between the control and 356043 treatment groups. Longissimus muscle area was approximately 4 cm<sup>2</sup> greater for pigs fed the control diets ( $P < 0.05$ ); however, when the raw *P*-value was adjusted using the adjustment for false discovery rate (FDR), this difference was not significant ( $P > 0.05$ ). Loin depth was greater for the control compared to the 356043 treatment group, even after the FDR adjustment ( $P < 0.05$ ). The reason for this difference is unclear, as the diets were similar in analyzed nutrient composition. However, all observed loin depth values for the 356043 group were



**Table 4. Growth data of pigs fed experimental diets.**

Item	Soybean meal source <sup>1</sup>			<i>P</i> -value	Adjusted <i>P</i> -value <sup>5</sup>	Commercial groups <sup>2</sup>		Sex effects <sup>3</sup>		SEM
	Control	356043	SEM <sup>4</sup>			92M72	93B15	Barrows	Gilts	
Initial BW <sup>6</sup> , lb	48.9	48.3	0.88	0.62	0.99	48.5	48.9	48.1	49.2	0.66
Final BW, lb	259.9	259.9	5.51	0.99	0.99	244.3	257.3	266.8 <sup>a</sup>	243.8 <sup>b</sup>	3.75
ADG <sup>7</sup> , lb	2.07	2.07	0.04	0.94	0.99	1.92	2.05	2.14 <sup>a</sup>	1.92 <sup>b</sup>	0.04
ADFI <sup>8</sup> , lb	5.58	5.53	0.13	0.77	0.99	5.20	5.47	5.80 <sup>a</sup>	5.09 <sup>b</sup>	0.09
G:F <sup>9</sup> , lb/lb	0.371	0.375	0.005	0.50	0.99	0.369	0.375	0.370	0.375	0.003

<sup>1</sup>Soybean sources from which soybean meal was derived: Control = nontransgenic, near-isoline compared to 356043; 356043 = transgenic containing the event DP-356Ø43-5; 92M72 and 93B15 = commercially available, nontransgenic varieties.

<sup>2</sup>Commercial group values included for reference purposes only; the true comparison of interest is Control vs 356043.

<sup>3</sup>Sex effects: means with unlike superscript letters differ ( $P < 0.05$ )

<sup>4</sup>SEM = standard error of the mean

<sup>5</sup>Adjusted *P*-value is adjusted based on the false discovery rate

<sup>6</sup>BW = body weight

<sup>7</sup>ADG = average daily gain

<sup>8</sup>ADFI = average daily feed intake

<sup>9</sup>G:F = gain to feed ratio

**Table 5. Carcass characteristic data of pigs fed experimental diets.**

Item	Soybean meal source <sup>1</sup>			<i>P</i> -value	Adjusted <i>P</i> -value <sup>5</sup>	Commercial groups <sup>2</sup>		Sex effects <sup>3</sup>		SEM
	Control	356043	SEM <sup>4</sup>			92M72	93B15	Barrows	Gilts	
HCW <sup>6</sup> , lb	195.8	195.8	4.2	0.99	0.99	184.5	193.1	201.9 <sup>a</sup>	182.8 <sup>b</sup>	2.9
Dressing, %	76.41	76.67	0.53	0.62	0.88	76.19	76.46	76.74	76.13	0.46
LM <sup>7</sup> area, cm <sup>2</sup>	45.1	41.3	1.5	0.03	0.07	44.0	47.9	45.0	44.2	1.2
LM depth, cm	6.83	6.31	0.17	< 0.01	< 0.01	6.61	6.94	67.9 <sup>a</sup>	65.5 <sup>b</sup>	1.6
10th-rib fat, cm	1.94	1.77	0.12	0.34	0.56	1.81	1.78	2.07 <sup>a</sup>	1.58 <sup>b</sup>	0.08

<sup>1</sup>Soybean sources from which soybean meal was derived: Control = nontransgenic, near-isoline compared to 356043; 356043 = transgenic containing the event DP-356Ø43-5; 92M72 and 93B15 = commercially available, nontransgenic varieties.

<sup>2</sup>Commercial group values included for reference purposes only; the true comparison of interest is Control vs 356043.

<sup>3</sup>Sex effects: means with unlike superscript letters differ ( $P < 0.05$ )

<sup>4</sup>SEM = standard error of the mean

<sup>5</sup>Adjusted *P*-value is adjusted based on the false discovery rate

<sup>6</sup>HCW = hot carcass weight

<sup>7</sup>LM = longissimus muscle

within the tolerance intervals calculated from each sex from the commercial reference treatment groups. Therefore, these values would be considered to be similar to those of pigs fed typical commercial soybean meal diets.

Globally, there has been a nearly 80-fold increase in the amount of land that is used to produce transgenic crops between 1996 and 2009. Over 75% of the 90 million hectares planted for soybean production in 2009 utilized transgenic varieties of soybeans with the primary trait being herbicide tolerance. Earlier experiments, which investigated feeding high concentrations of glyphosate tolerant soybeans in non-practical diets in order to determine the fate of DNA-fragments, resulted in no differences in any measured parameters compared to nontransgenic isoline control soybeans. There have been previous experiments in which soybean meal derived from other herbicide

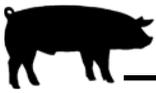
resistant varieties of transgenic soybeans have resulted in no differences among the measured traits compared to non-transgenic varieties when fed to swine. Other animal species, such as broilers and laying hens, also have been used to compare this herbicide tolerant soybean and soybean derived products with no statistical differences observed when compared to conventional soybeans. Other studies also have concluded that transgenic herbicide tolerant corn lines have very similar value as a feedstuff when compared to nontransgenic varieties. Previously developed herbicide resistant transgenic corn and soybean lines have been determined to be similar in composition to the nontransgenic conventional feedstuffs. There have been numerous studies designed to investigate potential problems associated with genetically modified crops in order to gain acceptance for their use. It is important that research continues to be

conducted assuring that these products are safe for use in today's agriculture.

## Conclusions

The results from this experiment agree with previous publications investigating the use of soybeans with glyphosate tolerance. In conclusion, the results obtained from this experiment document that growth data and carcass characteristics of pigs fed diets containing soybean meal produced from soybeans with the DP-356Ø43-5 event are similar to data obtained from pigs fed nontransgenic near-isoline and commercial soybean meal sources.

<sup>1</sup>Phillip S. Miller is a professor in the UNL Animal Science Department; David W. Rice, Brenda L. Smith, and Craig Sanders are scientists with Dupont/Pioneer; Roman Moreno and Justin W. Bundy are research technologists in the UNL Animal Science Department.



# Evaluation of Soybean Meal with the Genetically Modified Trait DP-3Ø5423-1 Fed to Growing Pigs

Soybean meal derived from soybeans with the modified DP-3Ø5423-1 trait does not adversely affect pig growth.

**Phillip S. Miller**  
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**Brenda L. Smith**  
**Craig Sanders**  
**Justin W. Bundy**  
**Roman Moreno<sup>1</sup>**

## Summary

*Nutritional equivalency of processed soybean meal from soybeans containing the gm-fad2-1 and gm-hra genes (event DP-3Ø5423-1; 305423) was evaluated in a feeding experiment with growing swine. A total of 64 pigs (initial BW = 60 lb) were randomly allotted based on BW and sex (gilts and barrows) to four dietary treatments consisting of standard corn-soybean meal-based diets formulated with nontransgenic near-isoline soybean meal (control), 305423 soybean meal, or soybean meal from nontransgenic commercial Pioneer varieties 92M72 or 93B15. A three-phase feeding program was used. Pigs were fed to an average final BW of 251 lb and harvested with standard carcass data collected. No differences were observed in final weight (250 vs 262 lb), ADG (2.1 vs 2.2 lb), or G:F (0.36 vs 0.36 lb/lb) between pigs fed diets containing the control and 305423, respectively. Also, no differences were observed for dressing percentage (78.6 vs 78.5%), LM area (45.4 vs 45.7 cm<sup>2</sup>), or 10th-rib backfat (2.0 vs 2.3 cm). Data generated from the commercial soybean meal groups were used to estimate experimental variability and to construct tolerance intervals for each parameter; estimate statements were used to compare values from pigs fed control diets and pigs fed 305423 diets. Individual growth and carcass trait values for the control and 305423 groups*

*were within the tolerance intervals calculated using the commercial soybean meals. Based on the results of this experiment, growth and carcass measures of pigs fed diets containing soybean meal produced from soybeans with the DP-3Ø5423-1 event are similar to that of pigs fed nontransgenic near-isoline and commercial soybean meal sources.*

## Introduction

It has become well known that the production of genetically modified crops continues to increase. Corn grain and soybean production make up a significant amount of genetically modified crops, globally. The genetic modification of 305423 soybeans results in an increased concentration of oleic acid and decreased concentrations of linoleic, linolenic, and to a lesser extent, palmitic acid in the soybeans. This modification produces high oleic phenotype soybeans. This trait is focused primarily on the characteristics of the oil produced from the soybeans.

The objective of this study was to compare the nutritional equivalency of 305423 soybeans by comparing the performance of swine fed diets containing 305423 soybean meal with those fed diets containing nontransgenic control soybean meal with comparable genetic background, or diets containing nontransgenic commercial soybean meal.

## Procedures

All procedures used in the experiment were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Nebraska–Lincoln (UNL). The study protocol was also reviewed and

approved by the Pioneer Animal Care and Use Committee.

## *Animals, Housing, and Experimental Design*

A total of 64 pigs (initial BW = 60 lb) were randomly allotted to four experimental groups based on weight and sex in a randomized complete block design. Pigs were housed in individual pens within a single environmentally controlled barn, which was split into two rooms with 32 pens located in each room.

## *Diets and Animal Data*

A three-phase feeding program was used with grower diets fed from approximately 55 to 130 lb; early-finisher diets fed from 130 to 200 lb; and late-finisher diets fed from 200 to 265 lb. Ingredient compositions of the diets are presented by phase in Table 2. All diets were formulated to meet or exceed the estimated requirements for all nutrients (NRC, 1998). Samples of each diet were collected for nutrient analysis (Moisture, protein, fat, GE, crude fiber, NDF, ADF, ash, Ca, P, and amino acids). Sub-samples of each diet were also submitted for PCR analysis to confirm the presence of the DP-3Ø5423-1 event in the 305423 diets and its absence from all control and reference diets.

Individual pig weights were recorded at the beginning and end of the experiment and also on day 14, 35 (phase change), 49, 63 (phase change), and 77. Diets were fed in a meal form, and both feed and water were provided ad libitum. Daily feed allocations were recorded and feed in the feeders was recorded at each feeding phase change. Growth data (ADG, ADFI, and G:F)



**Table 1. Analyzed composition of the soybean meals used in experimental diets (as-fed basis).**

Item	Soybean source <sup>1</sup>			
	Control	305423	92M72	93B15
Dry matter, %	96.10	93.50	95.10	95.70
Crude protein, %	49.50	48.50	50.90	49.50
Ether extract, %	1.60	1.60	2.10	1.40
GE <sup>2</sup> , Mcal <sup>3</sup> /kg	4.50	4.39	4.48	4.47
NDF <sup>4</sup> , %	16.10	9.30	10.10	12.30
ADF <sup>5</sup> , %	6.70	4.00	4.10	6.80
Calcium, %	0.33	0.30	0.31	0.37
Phosphorus, %	0.77	0.79	0.83	0.73
<b>Indispensable AA<sup>6</sup>, %</b>				
Arginine	3.64	3.58	3.65	3.59
Histidine	1.39	1.37	1.34	1.30
Isoleucine	2.52	2.32	2.42	2.39
Leucine	4.11	3.84	3.96	3.88
Lysine	3.15	3.09	3.17	3.15
Methionine	0.71	0.71	0.75	0.70
Phenylalanine	2.72	2.46	2.55	2.51
Threonine	1.99	1.90	1.97	1.89
Tryptophan	0.76	0.76	0.75	0.72
Valine	2.63	2.46	2.53	2.46

<sup>1</sup>Soybean sources from which soybean meal was derived: Control = nontransgenic, near-isoline compared to 305423; 305423 = transgenic containing the event DP-3Ø5423-1; 92M72 and 93B15 = commercially available, nontransgenic varieties.

<sup>2</sup>GE = gross energy

<sup>3</sup>Mcal = megacalorie

<sup>4</sup>NDF = neutral detergent fiber

<sup>5</sup>ADF = acid detergent fiber

<sup>6</sup>AA = amino acids

**Table 2. Ingredient composition of experimental diets (as-fed basis).**

Ingredient, %	Soybean meal source <sup>1</sup>			
	Control	305423	92M72	93B15
<b>Phase 1: 55 to 130 lb</b>				
Nontransgenic corn	70.416	70.986	72.585	70.530
Soybean meal	26.440	26.202	24.232	26.375
L-Lysine 98%	0.200	0.200	0.200	0.200
Limestone	1.158	0.837	1.199	1.093
Dicalcium phosphate	0.786	0.775	0.784	0.802
Salt	0.500	0.500	0.500	0.500
Vitamin-mineral premix	0.500	0.500	0.500	0.500
<b>Phase 2: 130 to 200 lb</b>				
Nontransgenic corn	77.897	77.008	77.686	76.804
Soybean meal	19.580	20.457	19.770	20.714
DL-Methionine 99%	0.016	0.011	0.008	0.012
Limestone	0.948	0.985	0.999	0.910
Dicalcium phosphate	0.559	0.539	0.537	0.560
Salt	0.500	0.500	0.500	0.500
Vitamin-mineral premix	0.500	0.500	0.500	0.500
<b>Phase 3: 200 to 265 lb</b>				
Nontransgenic corn	81.358	81.490	82.611	81.397
Soybean meal	16.272	16.112	14.954	16.262
L-Lysine 98%	0.003	0.016	0.043	0.002
Limestone	0.961	0.983	0.987	0.923
Dicalcium phosphate	0.406	0.399	0.405	0.416
Salt	0.500	0.500	0.500	0.500
Vitamin-mineral premix	0.500	0.500	0.500	0.500

<sup>1</sup>Soybean sources from which soybean meal was derived: Control = nontransgenic, near-isoline compared to 305423; 305423 = transgenic containing the event DP-3Ø5423-1; 92M72 and 93B15 = commercially available, nontransgenic varieties.

were calculated for the overall experimental period.

### Carcass Evaluation

Pigs were harvested over a period of three consecutive days. Harvest order was randomized among treatments within each harvest day. Live weight at slaughter and standard carcass data (HCW, 10th rib BF thickness, LMA, and loin depth measured at the 10th rib) were collected.

### Statistical Analysis

Data were analyzed using a mixed model ANOVA (PROC MIXED, SAS Institute Inc., Cary, N.C.). Treatment, sex, and the treatment × sex interaction were fixed effects in the analysis of growth and carcass data; room assignment was included as a random effect for growth data, and room assignment and harvest day were included as random effects for carcass data. The individual pig was the experimental unit for all data analysis.

Estimate statements were used to generate the true comparison of interest in this study (control vs 305423) for each measured trait. The observed P-values generated from the estimate comparison statement determined whether the mean of the control group was statistically different from the mean of the 305423 group; differences between means were considered significant if  $P < 0.05$ . False discovery rate (FDR) as described by Benjamini and Hochberg (1995) was applied across all traits analyzed to minimize the chance of falsely declaring a difference for a measured trait as significant when the difference may have occurred only by chance based on the number of measured traits. The FDR-adjusted P-value was reviewed if statistically significant differences ( $P < 0.05$ ) generated from the estimate comparison statement were observed for a trait. Data from pigs fed diets containing the commercial soybean meals (92M72 and 93B15) were used in the estimation of experimental variability. Specific comparisons between the commercial reference

(Continued on next page)



**Table 3. Analyzed nutrient composition of experimental diets (as-fed basis).**

Soybean source <sup>1</sup>	55 to 130 lb, phase 1				130 to 200 lb, phase 2				200 to 265 lb, phase 3			
	Control	305423	92M72	93B15	Control	305423	92M72	93B15	Control	305423	92M72	93B15
<b>Item</b>												
Dry matter, %	88.50	87.60	88.00	89.60	89.20	87.80	87.60	88.90	88.00	87.20	87.80	87.70
Crude protein, %	18.50	18.30	17.80	18.30	15.40	15.50	15.20	15.60	13.80	13.50	13.70	13.30
Ether extract, %	3.10	3.00	3.10	3.00	2.80	2.80	2.70	2.90	3.30	3.20	3.30	3.20
GE <sup>2</sup> , Mcal <sup>3</sup> /kg	3.90	3.88	3.90	3.91	3.86	3.83	3.86	3.88	3.87	3.81	3.85	3.85
Crude fiber, %	2.70	2.50	2.30	3.00	2.60	2.50	2.60	2.60	2.60	2.10	2.20	2.30
NDF <sup>4</sup> , %	11.30	10.40	10.90	10.90	10.50	9.50	11.10	10.80	11.90	11.20	11.40	10.80
ADF <sup>5</sup> , %	4.70	4.50	4.90	4.90	4.30	3.80	3.80	4.20	4.70	4.20	3.70	4.20
Ash, %	4.80	4.50	4.50	4.70	3.80	3.80	4.00	4.00	3.90	4.00	4.10	4.00
Calcium, %	0.83	0.65	0.69	0.74	0.60	0.58	0.65	0.59	0.59	0.59	0.58	0.57
Phosphorus, %	0.54	0.54	0.53	0.52	0.45	0.43	0.44	0.42	0.37	0.38	0.39	0.38
<b>Indispensable AA<sup>6</sup>, %</b>												
Arginine, %	1.17	1.22	1.19	1.16	0.92	1.00	0.97	0.99	0.87	0.88	0.82	0.86
Histidine, %	0.50	0.52	0.49	0.48	0.40	0.42	0.40	0.41	0.39	0.40	0.37	0.38
Isoleucine, %	0.82	0.81	0.79	0.78	0.63	0.65	0.61	0.66	0.60	0.57	0.57	0.59
Leucine, %	1.67	1.66	1.63	1.61	1.40	1.44	1.40	1.43	1.36	1.32	1.35	1.35
Lysine, %	1.10	1.15	1.14	1.12	0.75	0.82	0.79	0.81	0.70	0.71	0.65	0.70
Methionine, %	0.28	0.29	0.29	0.27	0.25	0.26	0.26	0.26	0.24	0.25	0.25	0.24
Phenylalanine, %	0.93	0.93	0.91	0.89	0.75	0.78	0.75	0.77	0.71	0.69	0.67	0.69
Threonine, %	0.66	0.67	0.67	0.65	0.55	0.58	0.57	0.56	0.53	0.52	0.50	0.51
Tryptophan, %	0.22	0.22	0.21	0.22	0.20	0.18	0.19	0.19	0.19	0.18	0.17	0.18
Valine, %	0.93	0.93	0.90	0.87	0.71	0.75	0.70	0.75	0.68	0.66	0.65	0.67

<sup>1</sup>Soybean sources from which soybean meal was derived: Control = nontransgenic, near-isoline compared to 305423; 305423 = transgenic containing the event DP-3Ø5423-1; 92M72 and 93B15 = commercially available, nontransgenic varieties.

<sup>2</sup>GE = gross energy

<sup>3</sup>Mcal = megacalorie

<sup>4</sup>NDF = neutral detergent fiber

<sup>5</sup>ADF = acid detergent fiber

<sup>6</sup>AA = amino acids

groups and control or 305423 groups were not generated from estimate statements. Instead, the reference group data were used to construct a 95% tolerance interval containing 99% of the observed growth and carcass trait values from pigs fed “typical” (non-transgenic commercial) soybean meal diets as described by Graybill (1976). If significant differences ( $P < 0.05$ ) remained after FDR adjustments were made, data from the control and 305423 groups were then evaluated to determine whether the observed values were contained within the tolerance interval; if so, that value was considered to be similar to feeding typical soybean meal. Tolerance intervals were generated by sex due to expected differences between the sexes.

## Results and Discussion

Analytical results of the soybean meal sources and the diets are shown in Tables 1, 2, and 3. Nutrient concentrations within each phase were similar among the treatment groups. Qualita-

tive real-time PCR analysis utilizing a primer and probe set specific for event DP-3Ø5423-1 confirmed the presence of the event in all three phases of the 305423 treatment diet, and its absence from all phases of control, 92M72, and 93B15 treatment diets.

Two mortalities were observed, one from each of the control and 354023 treatment groups. Necropsies performed determined that the cause of death was due to salmonella. Growth and carcass data of one pig from the control group was dropped from the final data sets prior to statistical analysis. This pig discontinued eating midway through the final diet phase, and despite a two-day treatment with antibiotics, feed intake for the remainder of the phase was much lower than that of the other pigs on study, and the pig lost weight.

Growth data results are presented in Table 4. Observed BW, ADG, and G:F were not different between pigs fed diets containing the control soybean meal and pigs fed diets containing the 305423 soybean meal. A treatment ×

sex interaction ( $P < 0.05$ ) was observed in the estimation of experimental variability for ADFI. Statistical contrast between the two groups of interest (control and 305423) demonstrated the treatment × sex interaction was not significant ( $P > 0.10$ ) between the two groups. The observed ADFI was greater ( $P < 0.05$ ) for the 305423 treatment group; however, this difference was not significant when the P-value was adjusted using False Discovery Rate ( $P > 0.10$ ). Additionally, all observed growth data values were within the tolerance intervals calculated for each sex using the data from the commercial soybean meal treatment groups (92M72 and 93B15). Sex differences ( $P < 0.05$ ) were observed for G:F with gilts being more efficient than barrows.

Observed carcass traits are presented in Table 5. Differences in hot carcass weights approached significance ( $P < 0.06$ ), with carcass weight being heavier for the 305423 treatment group; however, this difference was not significant when the P-value was adjusted using False Discovery Rate



**Table 4. Growth data of pigs fed experimental diets.**

Item	Soybean meal source <sup>1</sup>				Adjusted P-value <sup>5</sup>	Commercial groups <sup>2</sup>		Sex effects <sup>3</sup>		SEM
	Control	305423	SEM <sup>4</sup>	P-value		92M72	93B15	Barrows	Gilts	
Initial BW <sup>6</sup> , lb	59.3	59.5	0.22	0.63	0.85	59.3	59.3	59.3	59.3	0.22
Final BW, lb	249.8	261.7	5.29	0.12	0.23	253.5	243.6	256.8	247.4	3.75
ADG <sup>7</sup> , lb	2.07	2.20	0.07	0.13	0.23	2.12	2.01	2.14	2.05	0.04
ADFI <sup>8</sup> , lb	5.71	6.17	0.15	0.03	0.21	5.97	5.62	6.13	5.62	0.11
G:F <sup>9</sup> , lb/lb	0.362	0.358	0.01	0.67	0.85	0.356	0.356	0.351 <sup>b</sup>	0.364 <sup>a</sup>	0.009

<sup>1</sup>Soybean sources from which soybean meal was derived: Control = nontransgenic, near-isoline compared to 305423; 305423 = transgenic containing the event DP-3Ø5423-5; 92M72 and 93B15 = commercially available, nontransgenic varieties.

<sup>2</sup>Commercial group values included for reference purposes only; the true comparison of interest is control vs 305423.

<sup>3</sup>Sex effects: means with unlike superscript letters differ ( $P < 0.05$ )

<sup>4</sup>SEM = standard error of the mean

<sup>5</sup>Adjusted P-value is adjusted based on the false discovery rate

<sup>6</sup>BW = body weight

<sup>7</sup>ADG = average daily gain

<sup>8</sup>ADFI = average daily feed intake

<sup>9</sup>G:F = gain to feed ratio

**Table 5. Carcass characteristic data of pigs fed experimental diets.**

Item	Soybean meal source <sup>1</sup>				Adjusted P-value <sup>5</sup>	Commercial groups <sup>2</sup>		Sex effects <sup>3</sup>		SEM
	Control	305423	SEM <sup>4</sup>	P-value		92M72	93B15	Barrows	Gilts	
HCW <sup>6</sup> , lb	199.3	212.1	5.1	0.0501	0.21	204.8	193.8	205.9	199.1	4.0
Dressing %	78.65	78.52	1.23	0.78	0.85	78.48	77.56	78.34	78.26	1.20
LM <sup>7</sup> area, cm <sup>2</sup>	45.4	45.7	1.4	0.86	0.86	44.3	42.8	42.9 <sup>b</sup>	46.3 <sup>a</sup>	1.1
LM depth, cm	6.34	6.27	0.17	0.71	0.85	6.22	6.03	6.09	6.34	0.14
10th-ribfat, cm	1.96	2.33	0.17	0.08	0.21	2.43	1.94	2.34 <sup>a</sup>	1.99 <sup>b</sup>	0.14

<sup>1</sup>Soybean sources from which soybean meal was derived: Control = nontransgenic, near-isoline compared to 305423; 305423 = transgenic containing the event DP-3Ø5423-1; 92M72 and 93B15 = commercially available, nontransgenic varieties.

<sup>2</sup>Commercial group values included for reference purposes only; the true comparison of interest is control vs 305423

<sup>3</sup>Sex effects: means with unlike superscript letters differ ( $P < 0.05$ ).

<sup>4</sup>SEM = standard error of the mean

<sup>5</sup>Adjusted P-value is adjusted based on the false discovery rate

<sup>6</sup>HCW = hot carcass weight

<sup>7</sup>LM = longissimus muscle

( $P > 0.10$ ), and all values were within the calculated tolerance interval for each sex. Dressing percentage, LMA, loin depth, and 10th rib BF values were not different ( $P > 0.10$ ) between pigs fed control and 305423 treatment diets. Carcass differences ( $P < 0.05$ ) between sexes were as expected. Barrows had more BF than gilts, and gilts had a greater LMA.

The results obtained from this study are similar to previous research, which investigated genetically modified crops fed to livestock. The 305423 soybeans have been previously investigated using broilers and laying hens. In the broiler experiment, the hulls, soybean meal, and oil derived from 305423 soybeans were fed. In the laying hen experiment, only the soybean meal fraction from 305423 soybeans was fed. The results obtained from pigs fed soybean meal are

similar to the broilers fed components of 305423 soybeans and hens fed 305423 soybean meal. It was reported in the broiler study that no differences were observed in growth data, organ yield, and carcass data between the control and 305423 soybeans. In the laying hen study no differences were observed in egg production or egg quality between hens fed 305423 and hens fed control soybean meal. It was therefore concluded in the broiler and laying hen studies that 305423 soybeans were nutritionally equivalent to nontransgenic soybeans with similar genetics.

### Conclusions

Soybean meal from 305423 soybeans did not affect growth during the growing-finishing period. No differences were observed on growth or effi-

ciency of gain between pigs fed 305423 and the near-isoline nontransgenic soybean meal sources. Also, no differences were observed in carcass characteristics. All measured parameters were within the tolerance intervals derived from the commercially available soybean meal sources. In conclusion, the soybean meal derived from 305423 soybeans is equivalent to nontransgenic derived and commercially available soybean meal. Therefore, 305423 soybean meal can be fed to growing pigs without adverse effects on growth or carcass characteristics.

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# Genome-wide Association Studies of Sow Lifetime Productivity

Genome-wide association studies have potential to identify genetic markers that explain variation in sow lifetime productivity.

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## Summary

*Genome-wide association studies for identification of loci that influence sow lifetime productive and reproductive traits were performed in 639 gilts of two maternal crossbred lines. Variation in age at puberty was associated with the largest Single Nucleotide Polymorphisms (SNP) effects from all the reproductive traits. Clusters of SNPs associated with large effects on puberty onset were located on SSC1, SSC2, SSC10, SSC14, and SSC15. As expected, we found the variation of sow lifetime productivity traits to be potentially influenced by SNPs with relatively low effects located on many of the swine chromosomes. Clusters of SNPs clearly associated with major effects on lifetime number of live-born piglets were detected on SSC1, SSC2, SSC4, SSC13, SSC16, and SSC17. Combined analysis of the association results revealed loci that influence both reproductive and lifetime productivity traits.*

*This report describes our preliminary data of genome-wide association studies that explore the genetic variation responsible for sow reproductive and life productivity traits in a population that displays important reproductive and developmental differences. This unique population resource represents the foundation of multidisciplinary research that integrates genomics, nutrition, and reproductive physiology and aims to reduce culling rates, sow death losses, and enhance the productive life*

*of sows. We are in process of expanding the population in order to increase the power and accuracy in estimating SNP effects and the validation success of the research in commercial populations. The final goal of this research is to assemble a panel of DNA markers associated with sow reproductive and lifetime productivity and identify procedures to apply the information in whole genome selection.*

## Introduction

Sow infertility, death losses, and poor health, which lead to early culling and high replacement rates are major economic and welfare problems for the swine industry. The length of sow productive life is characterized by large phenotypic variance, moderate heritability, and as a result, substantial genetic variation is expected to exist in most populations. This trait is complex and believed to be affected by many genes with relatively small to moderate effects. As a consequence, the response to traditional selection methods is low because generation intervals will be long and accuracy of identifying genetically superior young animals is reduced. Therefore, this trait is one for which application of genomic tools will be especially helpful. The main objective of our research is to identify Single Nucleotide Polymorphism (SNP) markers and combinations of markers associated with sow lifetime productivity by conducting a genome-wide characterization of two maternal crossbred lines that differ in rate of lean growth, litter size, and lifetime production.

## Material and Methods

### Resource Population

Gilts originated from two genetic line combinations that differ in lean

growth and reproductive rates. Dams of the gilts were either a rotational cross of commercial Landrace x Large White or Nebraska Index Line sows inseminated with semen from boars of an unrelated industry maternal line to produce families of half-sib litters. Semen was collected and packaged from individual boars with known identities. Nebraska Index line was selected for increased litter size for 18 generations, and for increased litter size and within litter selection for increased growth and decreased backfat for the following generations. The gilts were produced in four replicates and selected randomly for this research at approximately 50 days of age. A total of 661 project gilts from 211 litters by 32 sires started the experiment at approximately 56 days of age.

### Nutrition

Gilts received the same diet and management until they reached an average age of 123 days. All of the gilts were placed on an experimental dietary regimen starting at 123 days of age until they were moved to the breeding barn. The diet was corn-soybean meal-based and formulated to contain 0.70% lysine, 0.70% Ca, and 0.60% P. The rest of the nutrients met or exceeded requirements for developing gilts. Half of the gilts representing both dam lines were provided ad libitum access to feed during the entire period. The other half of the gilts were placed on a restricted feeding regimen receiving a daily allotment of feed by weight that was 75% of that consumed by gilts on the ad libitum regimen. The restricted feeding regimen diet was fortified with all nutrients (except Se) to ensure that only energy intake was limited. During the breeding period and afterward, all the sows were subjected to the same diet.



## Phenotypes

Body weight was recorded at two-week intervals from 56 days of age and until breeding. Backfat and longissimus dorsi muscle area (LMA) were measured at two-week intervals from 123 to 230 days of age. Estrus detection was initiated at 140 days of age by moving gilts once daily from their pen to an adjacent room and exposing them to a boar for 15 minutes. This process continued until gilts were moved to the breeding barn or until all gilts from a pen had been observed in estrus at least twice. The gilts were maintained through four parities. Females that did not conceive or farrow a litter and those with structural problems were culled. Number of live, mummified, stillborn and weaned pigs in each litter, and weight of live pigs at birth and weaning were recorded. Backfat and LMA of sows were recorded ultrasonically within three days of farrowing and at weaning.

## DNA Isolation and Genotyping

The DNA was isolated from tail and ear tissues using the DNeasy blood and tissue kit (Qiagen). DNA quantity and quality was assessed by NanoDrop Spectrophotometer (Thermo Scientific) and agarose electrophoresis. All 639 gilts in the experiment were genotyped using the Porcine SNP60K BeadChip (Illumina) that includes 62,183 SNP assays. Three of the samples with different levels of DNA degradation (absent, limited, and increased degradation) were submitted for genotyping in duplicates. GenCall data analysis software was used to assign quality scores (GeneCall) for each genotype. A GeneCall genotype quality score of 0.15 was used as a cutoff threshold for removing low quality genotypes, which left 59,608 SNPs to be utilized for further analysis.

## Statistical Analysis

Proportion of variance for each reproductive trait accounted for by the 59,608 SNPs was estimated using a Bayes C approach (GenSel software, Fernando and Garrick, 2009). The probability of a particular SNP to have an effect on a trait was set to 0.005. Line, replicate, and diet were used as covariates in the analyses.

## Results and Discussion

### Quality Control and Statistics of High-Density Genotyping

All 639 gilts that reached puberty before 240 days of age were genotyped using the Porcine SNP60K BeadChip. The SNPs assays incorporated in this chip were previously validated in common swine breeds. The genotyping call rate of the samples varied from 41.0 to 99.9% of the SNPs, with 97% of the samples having a genotyping call rate greater than 90% and 57.8% of the samples with a call rate of at least 99.5%. The level of DNA degradation was evaluated by electrophoresis and was found to influence the fraction of SNPs successfully genotyped. The samples with no or low level of DNA degradation had more than 99% of the SNPs successfully genotyped. Samples with advanced level of DNA degradation had 87% of the SNPs successfully genotyped.

The rate of DNA degradation affects the genotyping accuracy of the samples with different level of degradation submitted in duplicate. The number of discrepancies between called genotypes of the samples with absent and limited DNA degradation was negligible (0.008 and 0.007% respectively) compared to the samples with increased level of DNA degradation (0.1%). The genotyping call rate varied among SNPs, with 2,947 SNPs generating genotypes for all 639 samples. There were 94.8% of the SNPs that generated genotypes for at least 90.0% of the samples and 46.1% of the SNPs that generated genotypes for at least 99.5% of the samples. There were 3.6% of the SNP assays that did not generate any genotype.

### Genetic Diversity of the Resource Population

The porcine SNP60K BeadChip provides sufficient density to potentially identify markers or group of markers associated with the variation of important physiological traits. These SNPs uniformly cover the genome with an estimated space of 40 kb per SNP. The resolution offered by this swine SNP array is well within the resolution of linkage disequilibrium (LD) in swine. Recent research on linkage disequi-

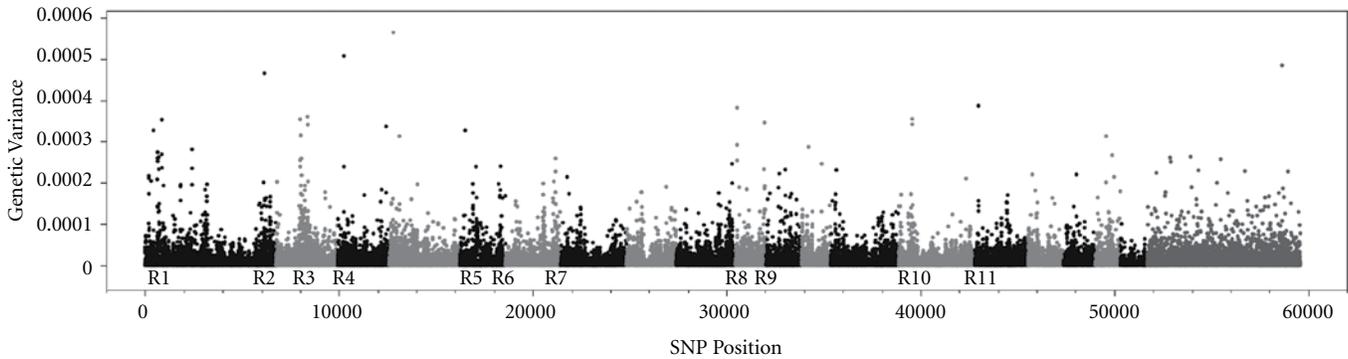
librium on multiple European breeds of swine suggests that SNP spacing should be of about 0.1cM. Therefore, a set of about 30,000 informative SNPs genotyped per individual is appropriate (1cM is approximately 1Mb) for genome-wide association studies in a reasonably large set of samples. The panel was optimized using multiple criteria for marker selection, including minor allele frequency (MAF) determined from representative sample sequencing, allele count, quality score, spacing, location, and validation status. The majority of the SNPs (55,427) are mapped on the latest assemble (build 9) of the porcine reference genome.

In the group of SNP assays with a great call rate (> 90%) there were 9,627 SNPs with a MAF of at least 0.05, 6,450 SNPs with a MAF of at least 0.01 and 4,147 SNPs completely fixed for one of the alleles. The average heterozygosity of the samples varied from 30.4 to 46.8%, with an average of 34%. Hardy-Weinberg equilibrium was calculated for each individual SNP using a chi-square test. There were 2,806 SNPs out of genetic equilibrium using Bonferonni correction for the cut-off *P* value of 8.7e-7.

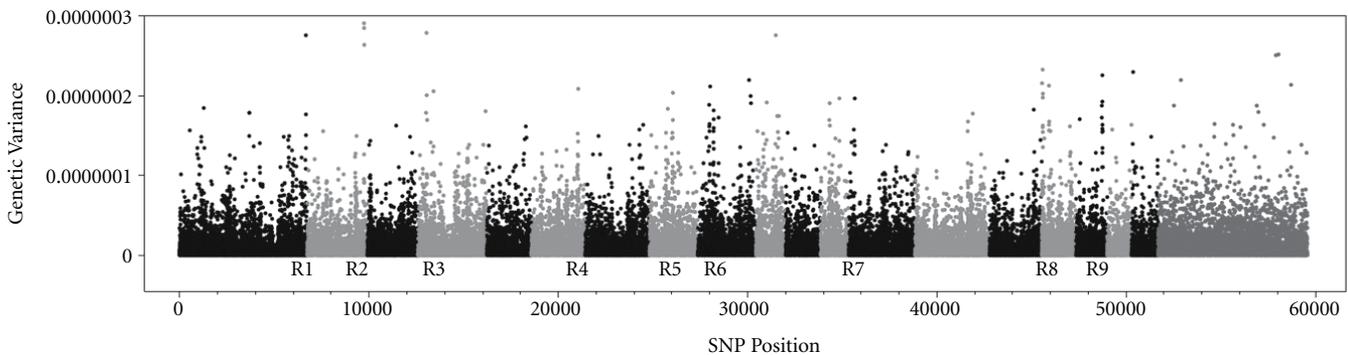
### Genome-wide Association Studies

A Bayesian analysis was conducted to estimate proportion of variance for each trait accounted for by the 59,608 SNPs from the Porcine SNP60K BeadChip. An early indicator trait of lifetime productivity is age at puberty (an early pubertal status is correlated with increased reproductive longevity of sows). Data generated by our group and others demonstrate clearly that genetic variation for age at puberty exists and is affected by energy intake during the pre-pubertal gilt development period. Variation in the age at puberty was associated with the largest SNP effects from all reproductive traits (Figure 1). This is not surprising since the heritability of age at puberty in our study was 0.42. Clusters of SNPs associated with large effects on puberty onset were located on Sus scrofa chromosome (SSC) 1 (21 to 29 Mb and 269 Mb regions), SSC2 (60 Mb), SSC10 (3 and 61 Mb), SSC14 (27 Mb),

*(Continued on next page)*



**Figure 1.** Genome-wide association analysis between 59,608 SNPs and age at puberty. Each dot represents the variance explained by a single SNP. The X axis represents the location of the SNPs on 18 autosomes and chromosome X. Y axis represents the contribution of that SNP to the genetic variance. Alternate black and grey colors represent the chromosomes, from SSC1 to 18, followed by chromosome X and by a group of SNPs (represented in grey) without a precise location. R1 to R11 represent regions with clusters of SNPs associated with high relative effects.



**Figure 2.** Genome-wide association analysis between 59,608 SNPs and the total number of live born pigs generated during sow lifetime. Alternate black and grey colors represent the chromosomes, from SSC1 to 18, followed by chromosome X and by a group of SNPs (represented in grey) without a precise location. R1 to R9 represent regions with clusters of SNPs associated with high relative effects.

and SSC15 (73Mb). Additional regions with clusters of SNPs associated with lower effects were also detected on two other regions of SSC1 and on SSC3, SSC5, and SSC6.

As expected, lower marker effects were identified for litter size from parity 1 to 4 (not shown) and lifetime productive traits (Figure 2). Variation of lifetime number of live-born piglets is affected by many environmental factors and potentially influenced by genes located on many chromosomes (Figure 2). Clusters of SNPs clearly associated with effects are located on SSC1 (291 Mb), SSC2 (132 Mb), SSC4 (144 Mb), SSC6 (108 Mb), SSC8 (62 Mb), SSC9 (22 and 123Mb), SSC13 (97 Mb), SSC16 (22 Mb), and SSC17 (56 Mb). Similarly, lifetime number of weaned piglets is affected by several chromosomes but clear clusters of SNPs associated with effects are located on seven chromosomes, each with a relatively low effect (not shown). Common

major loci that affect lifetime number of live-born and number of weaned piglets include those located on SSC1 and SSC17.

Age at puberty was negatively correlated with reproductive and lifetime productive performance in pigs ( $r = -0.18$ ). Given that both traits are dependent on the function of the hypothalamic-pituitary-gonadal axis, we anticipated that the variation of both traits would be influenced by the same gene variants. We found such evidence in a combined association analysis of age at puberty and sow lifetime production traits. For example, the same regions/genes from SSC1 potentially influence variation of both age at puberty and lifetime number of live-born piglets (Figure 1 and 2). Likewise, the same region detected on SSC1 also influenced lifetime number of weaned pigs (not shown). Interestingly, one of the clusters with the richest number of SNPs associated

with the total number of live-born and weaned pigs and located on the distal end of SSC17 also influenced litter size in parity 3.

Currently, we are in the process of moving the project closer to the final goal providing a small panel of markers associated with sow lifetime productivity by 1) expanding the size of the population resource in order to improve accuracy and detection power, and 2) designing validation protocols for markers associated with significant effects.

<sup>1</sup>Daniel C. Ciobanu is an assistant professor, Phillip S. Miller and Rodger K. Johnson are professors, Nichelle N. Ferdinand and Julie K. Tart are graduate students, and Autumn M. McKnite is a research technician in the UNL Animal Science Department; Stephen D. Kachman is a professor in the UNL Department of Statistics; Simon C. Brewer is a postdoctoral associate at the University of Wyoming.



# Variation in Response to Infection in Experimental Challenges with Porcine Circovirus 2b

Individual variation in the magnitude and time of immune response was demonstrated in experimental challenges with PCV2b.

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**Judith G. Galeota**  
**Timothy W. Moural**  
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## Summary

*Porcine Circovirus 2 is the etiological agent of many associated diseases that impact performance and increase mortality. Vaccination is costly and time consuming. In this study, 81 barrows of two commercial genetic lines were infected with a PCV2b strain and nine barrows receiving no inoculation were controls. Blood was drawn weekly and tested for levels of IgG, IgM, and viremia. Infected pigs showed three patterns of IgM response: early, late, and limited response. Pigs showing no response generally had faster growth and lower viremia, most likely due to an inhibition of virus replication. Early response individuals tended to have lower viremia and faster growth compared to individuals with late response. This important variation in immune response has a potential economic value and could be used in management practices and breeding programs. Future research will be focused on dissection of genetic and non-genetic factors explaining variation in immune response and disease susceptibility.*

## Introduction

Economic losses associated with susceptibility to Porcine Circovirus Associated Diseases (PCVAD) continue to have an impact on swine industry. Pigs affected by PCVAD display characteristics of wasting, diarrhea, interstitial pneumonia, dermatitis, lymphoid depletion leading to decreased immune responses, and susceptibility to other pathogens. Porcine Circovirus 2 (PCV2) is the causative source of PCVAD, but additional factors influence disease progression, of which host genetics and secondary immune stressors are highly important. Secondary infection with swine influenza, *Mycoplasma hyopneumoniae*, and Porcine Reproductive and Respiratory Syndrome (PRRS) can influence the severity and progression of PCVAD.

Vaccines for PCVAD exist, but they increase production costs, and producers who utilize the vaccine may still experience outbreaks. Even though only 5 to 15% of pigs infected with PCV2 display clinical symptoms, the whole herd must be immunized, which is a costly practice. Recent research has indicated that host genetics could influence susceptibility to disease. For example, several reports suggest that Landrace pigs are more susceptible to PCVAD than Pietrain and Duroc pigs. These breed differences mean that genetic variation within breeds likely exists and that selection for resistance may be possible. However, disease resistance is difficult to improve us-

ing traditional selection methods that require regular disease challenges or uniform and continued exposure to the pathogen. Selection based on DNA markers may be more effective. As long as marker panels with known relationships with immune response variables are available, selection can be practiced in any population without exposure to the pathogen. Before DNA selection can be implemented, comprehensive disease phenotypes and DNA samples need to be collected from pigs uniformly exposed to PCV2b to determine the relationships between DNA markers and disease susceptibility.

This study investigated individual response to experimental PCV2 infection by profiling major indicators of immune response in pigs from two crossbred lines. This study represents initial efforts to establish a large collection of samples and PCVAD phenotypes that will be used to identify genes and DNA markers associated with PCVAD resistance.

## Materials and Methods

### *PCV2b Isolate*

Isolate UNLVBMS was recovered from a pig that had symptoms characteristic of PCV2b infection. Viral DNA was isolated using QIAamp DNA Minikit (Qiagen). Two pairs of primers were used for amplification of the entire PCV2b genome. DNA amplification was performed using GoTaq Flexi DNA Polymerase (Promega)

*(Continued on next page)*



and PCR products were purified using ExoSAP-IT (USB Corporation). The viral DNA was sequenced from both directions using dye terminators and ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

### Animals and Facility

Animal use and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Nebraska–Lincoln (UNL). The experiment included 91 crossbred barrows originated from 24 litters that were either Large White x Landrace (W x R, n = 72) or a three-way cross with Duroc sires and W x R dams [D (W x R), n = 19]. Pigs were born at the UNL Swine Farm and at approximately 35 days of age were transported to the animal science research facility where the disease challenge was conducted. Pigs at the farm are routinely tested for major pathogens and are known to be negative for PRRSV. The pigs were housed in one room and randomly allocated to 18 identical pens with a combination of slatted and solid surface flooring. The pens provided approximately 0.65 square meters of floor space per pig. All pigs were fed ad libitum using a standard balanced diet.

### Experimental Infection

The objective was to infect pigs with virus after maternal antibodies had waned and before natural infection had occurred. Blood was drawn from pigs before infection and analyzed for IgG (maternal antibodies) and IgM (self-antibodies produced in response to infection) to determine when to initiate infection with PCV2b. The sample-to-positive (S/P) ratios of the maternal antibodies (IgG) in all individuals selected for the experiment were less than 0.3 at the time of inoculation, the level specified by the Ingezim ELISA protocol, which indicates that protection from maternal antibodies had waned. S/P ratios of IgM were less than 0.4, indicating that natural infection had not occurred.

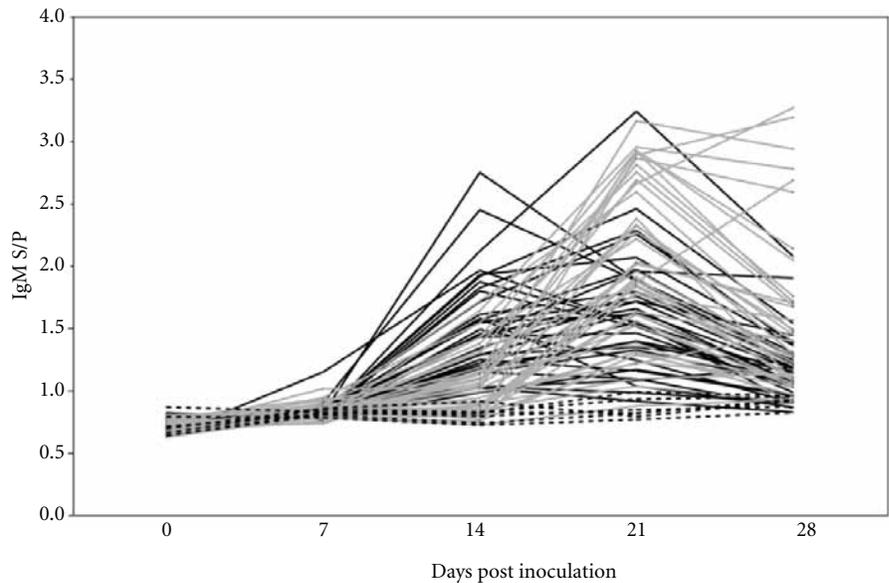


Figure 1. Individual IgM sample to positive ratio (S/P) profile during PCV2b experimental infection<sup>1</sup>.

<sup>1</sup>Infected individuals display different patterns of response to infection as 1) early — solid black; 2) late — solid grey; and 3) limited response — dashed black.

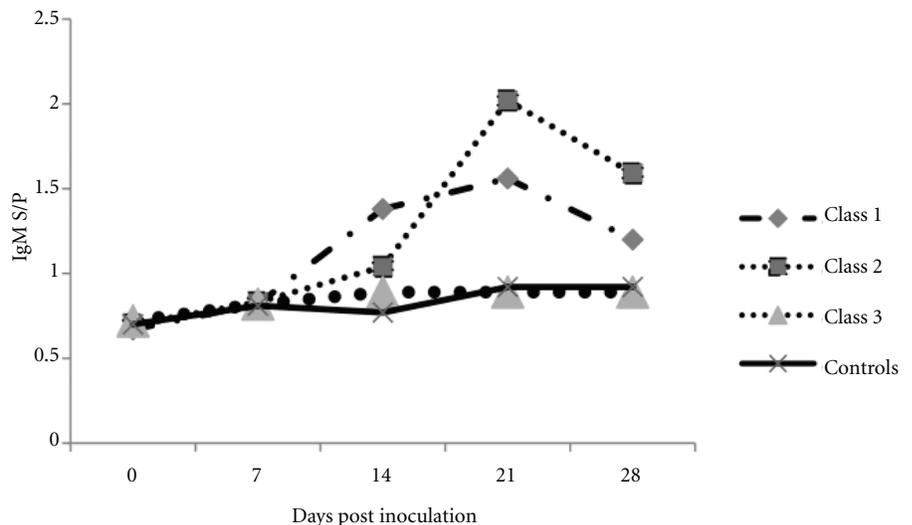


Figure 2. Least Square Means of IgM sample to positive ratios (S/P) during PCV2b experimental infection. Group 1 denotes pigs showing an early IgM response; Group 2 denotes pigs showing a late IgM response; Group 3 denotes pigs showing limited IgM response.

The virus inoculum contained the titer of  $10^{4.0}$  50% tissue culture infection dose (TCID<sub>50</sub>)/mL in minimum essential media with 50 ug/mL gentamicin and 5% fetal calf serum. The original experimental protocol was to infect each pig with 2 mL of inoculum intramuscularly (IM) and 3 mL intranasally (IN). However, approxi-

mately 20 minutes after inoculation of the first 20 pigs, anaphylactic shock occurred and eight of the pigs died. The dose administered to the remaining pigs was therefore altered. Most of the pigs (n = 58) received 1 mL IM and 3 mL IN. The rest of the pigs received a variation of this dose: 1 mL IM with 0.5 mL IN (n = 4) or 2 mL



**Table 1. Least Squares Means of the parameters profiled during challenge in the groups of individuals that showed different patterns in immune response to infection<sup>1</sup>.**

Trait	Group			
	1 (n = 33)	2 (n = 40)	3 (n = 7)	Control (n = 9)
ADG, 0 to 7 d.p.i. <sup>2</sup>	0.21	0.15	0.18	0.21
ADG, 7 to 14	0.36	0.32	0.37	0.37
ADG, 14 to 21	0.49	0.43	0.47	0.55
ADG, 21 to 28	0.59	0.53	0.63	0.53
ADG, 0 to 28	0.42 <sup>a</sup>	0.36 <sup>b</sup>	0.43	0.42
IgG 0	0.76	0.79	0.68	0.83
IgG 7	0.75 <sup>e</sup>	0.78 <sup>c</sup>	0.75 <sup>c</sup>	0.94 <sup>f,d</sup>
IgG 14	0.68 <sup>a</sup>	0.61 <sup>b</sup>	0.61	0.69
IgG 21	1.71 <sup>c,a</sup>	1.83 <sup>c,a</sup>	0.91 <sup>b</sup>	0.70 <sup>f,d</sup>
IgG 28	1.89 <sup>g,e</sup>	2.08 <sup>g,i</sup>	0.74 <sup>f,j</sup>	0.56 <sup>h</sup>
Viremia 7	4.56 <sup>e</sup>	4.37 <sup>c</sup>	3.85	3.22 <sup>f,d</sup>
Viremia 14	5.16 <sup>g,a</sup>	5.24 <sup>g,a</sup>	4.27 <sup>g,b</sup>	2.13 <sup>h</sup>
Viremia 21	5.8 <sup>g,c</sup>	6.17 <sup>g,e</sup>	4.67 <sup>f,d</sup>	3.79 <sup>h</sup>
Viremia 28	4.66 <sup>g</sup>	4.7 <sup>g,a</sup>	3.96 <sup>c,b</sup>	2.56 <sup>h,d</sup>
Viremia AUC	124.11 <sup>g,d</sup>	127.53 <sup>g,d</sup>	104.06 <sup>c</sup>	72.991 <sup>h,d</sup>

<sup>1</sup>The infected individuals were separated in groups based on their response to infection as 1) early, 2) late, and 3) limited response to infection.

Significant differences between groups are represented with different superscripts in the same row as follows: <sup>a,b</sup>  $P < 0.05$ , <sup>c,d</sup>  $P < 0.01$ , <sup>e,f</sup>  $P < 0.001$  and <sup>g,h</sup>  $P < 0.0001$  or <sup>i,j</sup>  $P < 0.0001$ .

<sup>2</sup>d.p.i = days post inoculation; units for ADG are kilograms of gain per day; units for IgG are sample-to-positive ratio; units for viremia are base 10 logarithm of the viral DNA copies per mL of serum.

**Table 2. Correlations between average daily weight gain (ADG) and viral load. Moderately negative correlations were estimated between cumulative weekly measures of area under the curve (AUC) for viremia and ADG during the entire challenge (-0.22 to -0.29).**

Traits	ADG	ADG	ADG	ADG	AUC	AUC	AUC	AUC
	0 to 7	0 to 14	0 to 21	0 to 28	0 to 7	0 to 14	0 to 21	0 to 28
ADG 0 to 7	1.00	0.83	0.74	0.66	-0.01	-0.02	-0.03	-0.04
ADG 0 to 14	0.83	1.00	0.88	0.82	-0.12	-0.12	-0.11	-0.12
ADG 0 to 21	0.74	0.88	1.00	0.92	-0.17	-0.17	-0.18	-0.20
ADG 0 to 28	0.66	0.82	0.92	1.00	-0.22 <sup>a</sup>	-0.24 <sup>a</sup>	-0.27 <sup>a</sup>	-0.29 <sup>b</sup>
AUC 0 to 7	-0.01	-0.12	-0.17	-0.22	1.00	0.96	0.84	0.78
AUC 0 to 14	-0.02	-0.12	-0.17	-0.24	0.96	1.00	0.95	0.90
AUC 0 to 21	-0.03	-0.11	-0.18	-0.27	0.84	0.95	1.00	0.99
AUC 0 to 28	-0.04	-0.12	-0.20	-0.29	0.78	0.90	0.99	1.00

<sup>a</sup> $P < 0.05$  <sup>b</sup> $P < 0.01$

IM with 0.5 mL IN (n = 8). The pigs used for these treatments were chosen at random. Ten pigs were selected as negative controls, assigned to separate pens, and not inoculated.

### Serology

Blood samples were collected before inoculation and at 7, 14, 21, and 28 days post inoculation (d.p.i.). Levels of PCV2 specific antibodies, IgG and IgM, were measured from serum using ELISA assays (Ingenasa). Samples were considered positive if the calculated S/P ratio was greater than 0.3 for IgG and 0.4 for IgM. Antibody data were

normalized based on positive control values obtained for each plate.

### Clinical Evaluation and Necropsy

Pigs were observed daily for clinical signs of infection, and weighed at 0, 7, 14, 21, and 28 d.p.i. Necropsy was performed at 28 d.p.i. Lung, spleen, and mesenteric and bronchial lymph nodes were collected for histology examination and gene expression analyses.

### PCV2 Quantification

Viral DNA was extracted from serum collected at 7, 14, 21, and 28

d.p.i. using QIAamp DNA Minikit (Qiagen). Estimates of the number of viral copies were obtained by quantitative real-time PCR using TaqMan Master Mix and ABI 7900 Real Time PCR System (Applied Biosystems). The area under the curve (AUC) was calculated to estimate total viral load throughout the 28-day experiment.

### Statistical Analysis

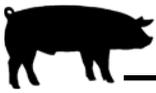
Least Square Means (LSM) were obtained using mixed-model procedures including the immune response pattern with crossbred lines as fixed effects and pen and litter as random effects. Analysis of the IgM antibody profiles during the challenge revealed that infected individuals are characterized by three patterns of immune response and could be separated in three groups: 1) individuals that responded immediately to infection with the highest change in specific PCV2 IgM antibodies from 7 to 14 days post infection (d.p.i.) (n = 33); 2) individuals that responded late with the greatest change from day 14 to 21 (n = 40); and 3) individuals that did not respond to infection (n = 7) (Figure 1). Most pigs clearly fit into one of these groups, but in some cases pigs were placed in a group based on a subjective judgment. Correlations among traits were calculated from variances and covariances adjusted for line effects.

## Results and Discussion

### Genetic Characterization of the PCV2b Isolate

The PCV2b strain utilized in this experiment was isolated from a pig showing clinical symptoms of PCVAD. The viral DNA was isolated and sequenced having the highest genetic similarity with the PCV2b strain FMV-05-6507. This strain was first identified in 2005 in Quebec, Canada, and is known to induce clinical signs of postweaning multisystemic wasting syndrome (PMWS, a form of PCVAD) and increased mortality rate.

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### Anti-PCV2-IgM and -IgG Antibodies

All candidate pigs for the PCV2b challenge were produced by dams that were vaccinated for PCVAD. The offspring receive anti-PCV2 antibodies via colostrum that provides immunity against this pathogen. Its effectiveness depends on the rate of antibody decay that varies from 5 to 21 weeks. Seventy-five percent of the 120 five-week old candidate pigs for experimental challenge had levels of maternal antibodies (IgG) below the threshold (S/P ratio lower than 0.3) that differentiate PCV2 negative from positive pigs. Eighty-two percent of the W x R pigs (n = 89) had IgG levels below the threshold compared to 58% of the D (W x R) pigs (n = 31). The levels of IgG in the two crossbred groups did not differ ( $P > 0.10$ ) before infection.

With the exception of day 7, pigs in Group 3 had the lowest IgM values during the challenge. Observed IgM values calculated as sample to positive ratios for each group at 0, 14, 21, and 28 d.p.i. are presented in Figure 2. At day 0 and 7, IgM values were similar, and at day 14, the controls had the lowest value at 0.77, whereas the Group 1 pigs had the highest at 1.38. The IgM values of the pigs from Group 1 remained elevated at day 14, and then the response began to wane at day 21. The IgM values of Group 2 pigs reached the peak at day 21 and declined thereafter. At day 21, the controls and Group 3 pigs had the lowest IgM values, 0.92 and 0.89, respectively. At necropsy on day 28, the controls and Group 3 pigs continued to have the lowest IgM values, whereas Group 1 pigs had the highest and Group 2 had intermediate values.

Recent reports showed that rate of maternal IgG decay varies considerably between individuals and breeds, which affects infection outcome and disease progression. There were no differences between groups in IgG level at day 0 (Table 1). With the exception of day 7, Group 3 had the lowest IgG values during the challenge. Interestingly, the IgG values of all individuals from Group 3 were below the positive threshold. Differences in IgG levels

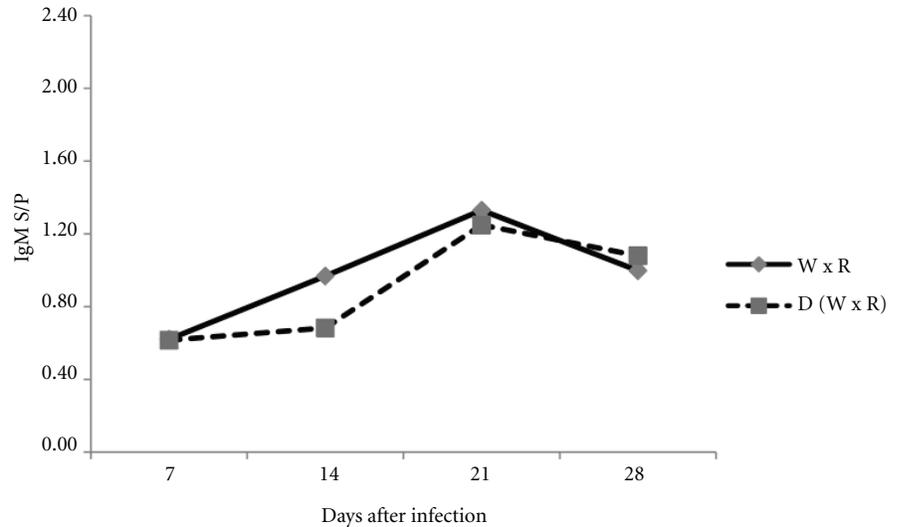


Figure 3. IgM sample to positive ratios (S/P) during the experimental challenge of two crossbred lines. Large White x Landrace pigs are denoted W x R, while Duroc (Large White x Landrace) pigs are denoted D (W x R).

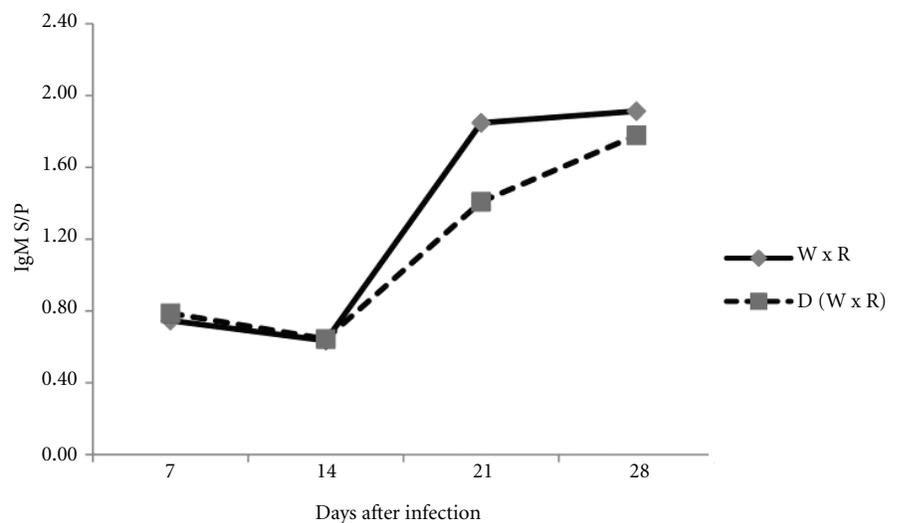


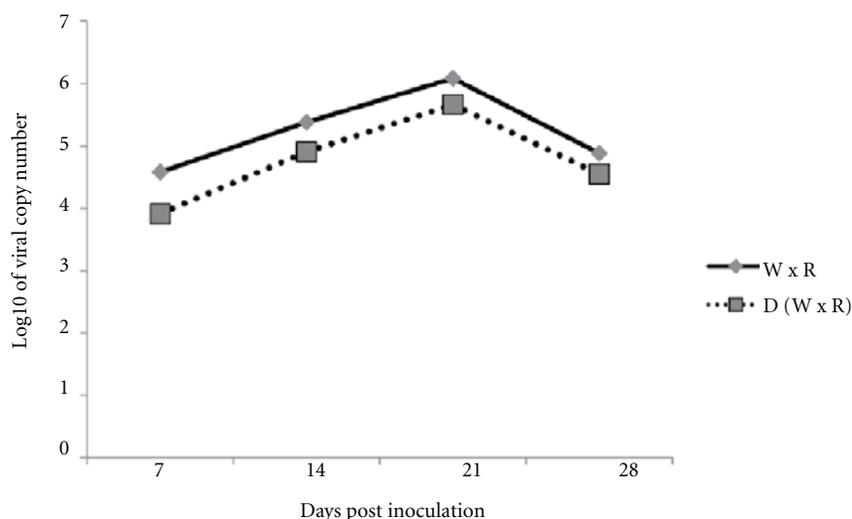
Figure 4. IgG sample to positive ratios (S/P) during the experimental challenge of two crossbred lines. Large White x Landrace pigs are denoted W x R, while Duroc (Large White x Landrace) pigs are denoted D (W x R).

between groups occurred on day 14, 21, and 28 ( $P < 0.05$ ). IgG values for infected and control pigs did not differ at day 0, averaging approximately 0.77. At day 7, the controls had the highest values at 0.94, whereas the other three groups remained steady around 0.75. At 14 d.p.i., the controls were lowest at 0.70 and the Group 2 pigs were the highest at 1.83. The same trend continued at 21 d.p.i. The IgG response in pigs in Group 3 was markedly different. Values remained low until day 14,

increased to a value of 0.91 at day 14, and then returned to baseline levels at day 28. There were no significant differences in IgM and IgG between the two genetic lines, W x R and D (W x R), at any time point (Figures 3 and 4).

### Amount of PCV2 DNA in Serum Samples

Following the experimental infection, quantitative PCR results for viral copy count showed that most of the



**Figure 5.** A comparison of PCV2b viral copy numbers in two crossbred lines. Large White x Landrace pigs are denoted W x R, while Duroc (Large White x Landrace) pigs are denoted D (W x R).

barrows at day 7, 14, 21, and 28 d.p.i. tested positive for PCV2 DNA (Table 1). Differences in viral copy number between W x R and D (W x R) existed at day 7 ( $P < 0.006$ ) and day 14 ( $P < 0.01$ ). DNA copy number increased in both lines through day 21 and then declined (Figure 5).

Pigs in Group 3 had fewer viral copies than pigs in the other groups. At day 7, Group 1 had the highest response at 4.56, whereas the response in Group 3 was 3.85. At day 14, 21, and 28, Group 2 had the highest viral copy count at 5.24, 6.17, and 4.7, respectively; Group 3 remained the lowest at 4.27, 4.67, and 3.96, respectively. For viremia AUC, the Group 3 pigs were the lowest, with Group 1 being intermediate and Group 2 being the highest. Viremia of the control pigs remained baseline throughout the study.

The amount of PCV2 administered to pigs did not influence disease progression, with the exception of viremia results at day 7 ( $P < 0.05$ ). However, there were only four pigs in the group that differed from the others, and they received the lowest viral dose of all 81 pigs infected.

### Clinical Evaluation

Only one death occurred during the four weeks of experimental challenge. This individual died during the last week of challenge, displayed wasting disease and had the highest viral load (AUC) in the first three weeks of the challenge. This individual displayed an early immune response and was inoculated with the lowest viral dose. The only difference in growth occurred between Groups 1 and 2 for average daily gain during the 28-day period ( $P < 0.05$ ). However, consistent with viremia and IgM responses, pigs in Group 3, that had a limited immune response, had the greatest ADG.

### Correlations

Correlations between average daily gain and viremia results are summarized in Table 2. Moderately strong, negative correlation was estimated between average daily gain during the entire challenge and viral load (AUC) ( $r = -0.29$ ).

### Conclusion

The results of this preliminary research provide strong evidence of

significant variation in immune response in experimental challenges with PCV2b. Analysis of the profiles of viral load and antibody response revealed three groups of individuals that display important variation in response to infection. This variation is the source of the differences in growth between pigs during the challenge. The main difference between individuals from Group 1 and 2 was in the time of response, whereas the difference between these two groups and Group 3 was in the magnitude of response. The source of limited immune response in individuals from Group 3 is most likely a mechanism that inhibited virus replication. Individuals that responded immediately to infection (Group 1) had greater ADG than those that responded late (Group 2). Pigs in Group 3 had the greatest rate of growth during the 28-day period following infection, but the observed differences were not statistically significant.

The objective of our future research is to identify if the source of the differences is genetic and to uncover potential genes and genetic variants responsible for PCVAD susceptibility. This research will generate knowledge that can be applied to other viral diseases such as PRRSV. The long-term objective is to generate a panel of genetic markers that can be used in breeding programs to improve genetic resistance to PCVAD. The benefits of improving PCVAD susceptibility using genetic markers are obvious: lower production costs associated with improved robustness and a decrease in the number of vaccinated pigs, fewer welfare issues, and increased international competitiveness of the U.S. swine industry.

<sup>1</sup>Theresa P. Bohnert is a graduate student, and Autumn M. McKnite is a research technician in the UNL Animal Science Department; Judith W. Galeota is research manager, Timothy Moural, research technologist, and Seth Harris, assistant professor in the UNL School of Veterinary Medicine and Biological Sciences; Rodger K. Johnson is a professor, Thomas E. Burkey and Daniel C. Ciobanu are assistant professors in the UNL Animal Science Department.



# The Effect Of Dam Parity On Milk Yield

Preliminary experiment indicates that dam parity may not affect milk yield.

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## Summary

Previous research results indicate that dam parity may affect concentrations of immunoglobulin (Ig) G in serum of progeny derived from parity (P) 1 or 4. The observation that transfer of passive immunity may be affected by dam parity has led to the hypothesis that the differences in Ig between dam parity could be a result of differences in milk yield. The objective of this study was to compare milk yield between P3 and P1 dams. The weigh-suckle-weigh technique was used where piglets were weighed, allowed to suckle, then weighed again. Ten dams from each parity were utilized. No differences were observed in milk yield between parities. More dams may be needed to observe differences in milk yield.

## Introduction

Currently, research is being conducted at the University of Nebraska–Lincoln (UNL) to evaluate the effect of dam parity on progeny growth performance and health status. Results from this research indicate that progeny derived from parity (P) 4 dams have increased ( $P < 0.02$ ) circulating IgG compared to progeny derived from P1 dams in blood samples collected during the first two weeks post-farrowing. However, when IgG concentrations were quantified in colostrum and milk samples obtained from P4 and

P1 dams during the same period, no differences between dam parity were observed. Thus, we hypothesized that differences in circulating IgG between P4 and P1 progeny may result from either a greater production (i.e., increased volume) of colostrum/milk by the dam, and/or greater consumption of colostrum/milk by P4 progeny. Therefore, the objective of this preliminary study was evaluate milk yield between P1 and P3 dams.

## Materials and Methods

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at UNL. Milk yield measurement were recorded from P1 and P3 dams ( $n = 10$  dams per parity) on day 10 of lactation. To measure yield, the following procedures were implemented. All

Table 1. Effects of dam parity on milk yield.

	Number	Yield, g	SEM <sup>a</sup>	P-value
P1	10	36.17	5.33	0.493
P3	10	41.34	5.31	

<sup>a</sup> Standard Error of the Mean

piglets within a litter were removed from the dam and placed in large plastic tubs with heat lamps for 55 minutes. Piglets were then moved to cold, wet concrete for five minutes to encourage urination and defecation. Individual pig weights were taken before returning to the dam (presuckle weight). Piglets were quickly weighed after termination of suckling (post-suckling weight) before urination or defecation. On average, after returning piglets to the dam, piglets were allowed to suckle for 15 min. If piglets urinated

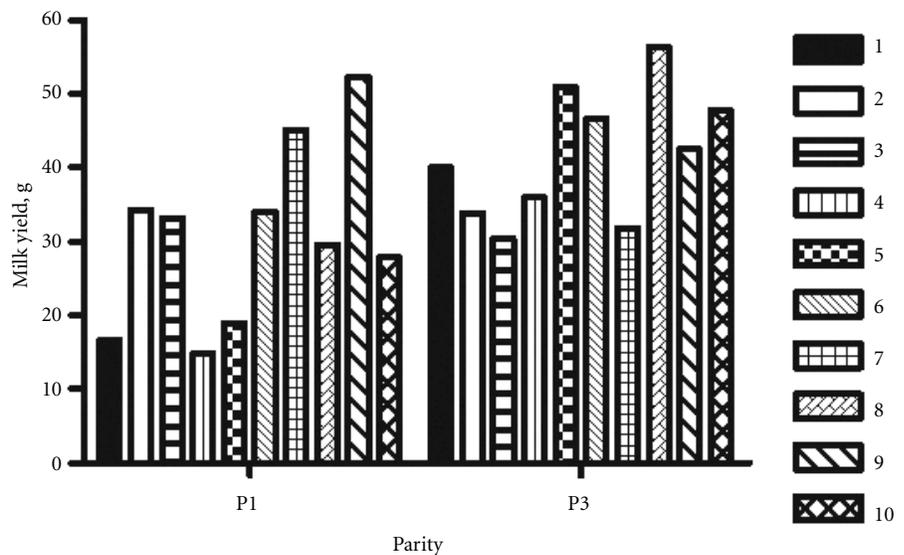


Figure 1. The effect of dam parity on milk yield (g). Each bar represents the least square mean of each litter (1 – 10).



or defecated before weighing, a paper towel was used to collect the excrement and weighed with the pig. This process was repeated three times (total of four repetitions/litter). Milk yield was recorded as the difference between presuckling weight and postsuckling weight of each individual piglet in the litter.

The MIXED procedure of SAS was used to analyze milk yield. The model included terms for fixed effects of parity. Random statements included repetitions nested within pig and pig nested within litter. The main effect of dam parity was detected using the least significant difference procedure. All means presented are least square means.

## Results and Discussion

Milk yield results are presented in Table 1. Milk yield was not affected by dam parity ( $P = 0.493$ ). However, milk yield in P3 dams was numerically greater compared to P1 dams.

Previous studies on milk yield have reported on a large number of dams due to a high probability for error in collecting milk yield based on differences in presuckling and postsuckling piglet weight. Therefore, the results of this study may be confounded by the low number of dams utilized. It is therefore difficult to determine if parity effects dam milk yield.

## Conclusion

Dam milk yield is not affected by parity. However, an increased number of dams may be needed to determine more accurate effects of parity on milk yield.

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<sup>1</sup>Erin E. Hinkle and Huyen Tran are graduate students and Justin W. Bundy is a research technologist; Phillip S. Miller is a professor and Thomas E. Burkey is an assistant professor in the UNL Animal Science Department. Matthew W. Anderson is manager and Jeffrey M. Perkins is a research technician at the UNL Swine Research Farm.



# Effect of Dam Parity on Litter Performance, Passive Immunity, and Fecal Microbial Populations (Parity 1 vs. 3)

Increased parity increases litter performance and passive immunity.

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**Justin W. Bundy**  
**Matthew W. Anderson**  
**Jeffrey M. Perkins**  
**Phillip S. Miller**  
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## Summary

*Previous research has shown that parity (P) 4 progeny have greater weaning weights and advantages related to transfer of passive immunity compared to P1 progeny. The objective of this experiment was to evaluate litter performance, passive immunity, and fecal microbial populations among P1 and P3 dams and their progeny. No differences were observed between parities in total born, live-born, mummies, deaths, or total weaned; however, P3 dams had a greater number of stillborns ( $P < 0.013$ ) compared to P1 dams. Litter BW was increased for P3 litters on day 0, 7, 14, and at weaning (day 16) compared to P1 dams ( $P < 0.001$ ). Dam serum IgG concentrations on day 114 of gestation were increased ( $P < 0.001$ ) for P3 dams compared to P1 dams. Parity  $\times$  day interactions were observed for progeny IgG ( $P < 0.01$ ) and IgA ( $P < 0.001$ ) concentrations. Progeny derived from P3 dams had greater concentrations IgG on day 0 and 14 compared to P1 progeny, and IgA concentrations were greater in P3 progeny on day 0. Litter performance and transfer of passive immunity may be affected by dam parity.*

## Introduction

Previous research conducted at the University of Nebraska–Lincoln (UNL) has indicated that growth performance, transfer of passive immunity, and gastrointestinal microbial populations may be affected by dam parity. Results from previous work indicate that Parity 4 progeny were heavier at birth and at weaning, which corresponded with increased concentrations of progeny serum IgG. Microbial populations were affected by parity with P4 piglets having an increased amount of diversity (number of species and abundance). However, studies by Mahan et al., reported greater differences in parity existing between P1 and P3. Therefore, the objective of this study was to evaluate litter performance, passive transfer of immunity, and microbial populations between P1 and P3 dams and their progeny.

## Materials and Methods

### Experimental Design

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Nebraska–Lincoln. Dams utilized in the current study included first parity dams (P1;  $n = 56$ ) and fourth parity dams (P4;  $n = 49$ ) that all farrowed over a 22-day period. The dam and litter performance parameters recorded included: Number of pigs/litter (total born, born live, stillbirths, mummified

fetuses, pigs weaned, and pre-weaning mortality), litter weight at birth (LBW), and litter weight at weaning (LWW). All piglets from each litter were weighed on day 0, 7, 14, and at weaning (day 19).

### Laboratory Analysis

Blood samples were collected from all sows via jugular venipuncture at two time points during gestation (day 90 and 114) and at a final time point immediately following parturition (day 0). During lactation, samples were obtained at day 0 (colostrum), 7 (mid-lactation), and 14 (late-lactation) from each functional teat. For mid- and late lactation milk collection, oxytocin was administered to facilitate milk collection. Colostrum and milk samples were diluted (1:50,000) and concentrations of IgA and IgG were quantified as described below. Blood samples were collected from six piglets from each litter on day 1, 7, and 14. Serum was harvested by centrifugation (20 min at  $1,500 \times g$ ) and frozen for subsequent analyses. Concentrations of immunoglobulins (IgA and IgG) in serum, colostrum, and milk were quantified via swine-specific enzyme-linked immunosorbent assays (ELISA; Bethyl Labs Inc., Montgomery Tex.).

### Statistical Analysis

The MIXED procedure of SAS was used to analyze the progeny serum and lactation data as a completely random design with repeated measures over time on each experimental unit.



**Table 1. Treatment effects of sow parity on litter and pig measurements.**

Item	Parity		SEM <sup>a</sup>	P - value
	1	3		
No. of sows	56	49		
Pigs, no./litter				
Total born	12.57	13.26	0.80	0.390
Born live	11.66	11.89	0.73	0.747
Stillbirths	0.50	1.00	0.19	0.013
Mummies	0.41	0.36	0.13	0.748
Mortality	1.91	2.14	0.33	0.496
Weaned	9.57	9.73	0.57	0.777
<b>Litter wt, lb</b>				
Birth	32.17	40.62	1.31	0.001
Day 7	50.70	63.73	1.83	0.001
Day 14	85.78	105.81	2.95	0.001
Weaning (day 16)	96.48	126.14	3.79	0.001

<sup>a</sup>SEM = Standard error of the mean

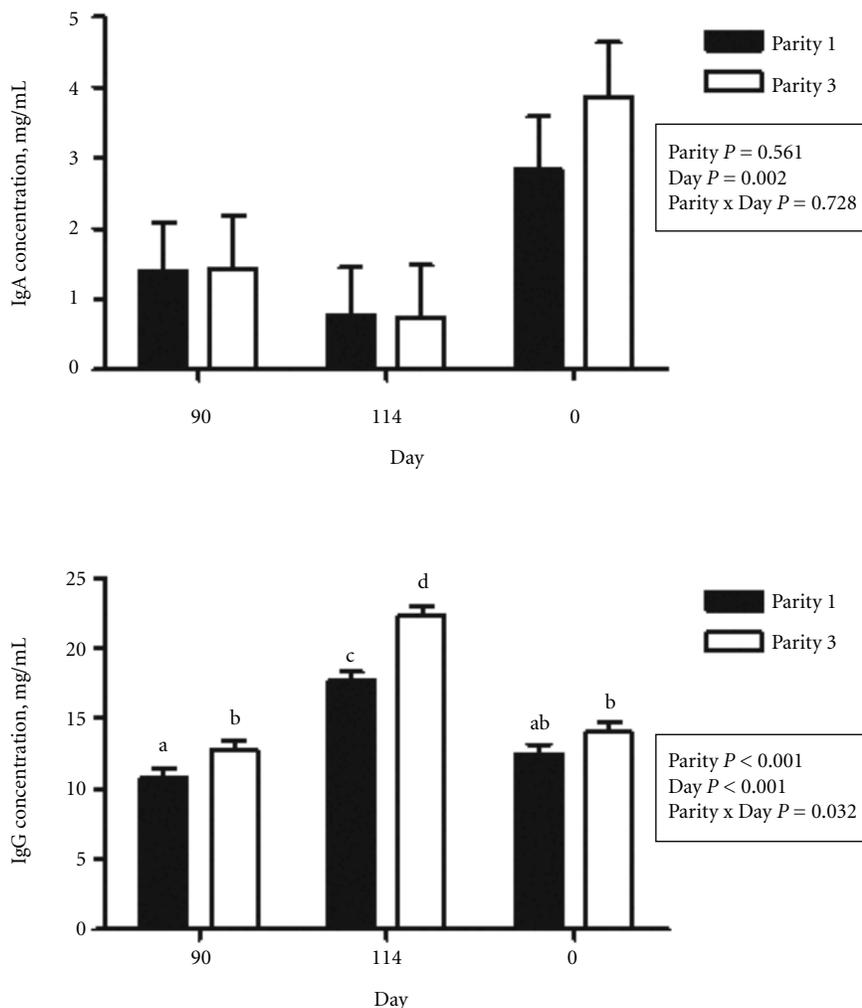
The model included terms for the fixed effects of parity and time and their interaction. Comparisons between parity and time were made only when a significant ( $P < 0.05$  unless noted otherwise) F-test for the main effect or interaction was detected using the least significant difference procedure. All means presented are least squares means. Litter performance data was analyzed using the MIXED procedure of SAS as a completely randomized design.

## Results and Discussion

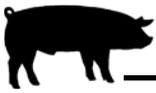
Dam and litter performance is presented in Table 1. No differences were observed between parities in total born, live born, mummies, deaths, or total weaned; however, P3 dams had a greater number of stillborns ( $P < 0.013$ ) compared to P1 dams (1.0 and 0.5 pigs, respectively). Litter body weight was increased for P3 litters on day 0, 7, 14, and at weaning (day 16) compared to litters derived from P1 dams ( $P < 0.001$ ).

Serum IgA and IgG concentrations for P1 and P3 dams are presented in Figure 1. A significant parity  $\times$  day interaction ( $P < 0.032$ ) was observed for dam serum IgG concentrations (Figure 1). On day 90 and 114, IgG concentrations in P3 dams were greater ( $P < 0.05$ ) compared to P1 dams. When averaged among both parities, IgG concentrations were greater ( $P < 0.05$ ) on day 114 compared to all other time points. No effects of dam parity were observed for circulating IgA in dams ( $P = 0.56$ ). However, as expected, a significant day effect was observed where circulating IgA concentrations were greater ( $P < 0.05$ ) on day 0 compared to all other time points. The concentration of Ig (A and G) in milk samples during lactation (day 0, 7, and 14) were not affected by dam parity ( $P > 0.40$ ; Figure 2). However, a significant effect of day was observed where greater ( $P < 0.001$ ) concentrations of Ig (A and G) were observed on day 0 com-

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**Figure 1. Circulating concentrations of IgA (top panel) and IgG (bottom panel) in parity (P) 1 and 3 dams. Immunoglobulin concentrations were evaluated in serum obtained at day 90 and 144 of gestation and immediately following parturition (day 0). Each bar represents the least squares mean ( $\pm$  SEM) of 56 and 49 observations for P1 and P3 dams, respectively.**



pared to all other time points when means were averaged among both parities.

Parity  $\times$  day interactions were observed for progeny IgG ( $P < 0.001$ ) and IgA ( $P < 0.001$ ) serum concentrations (Figure 3). The concentration of Ig (A and G) in serum from P1 and P3 progeny were greater ( $P < 0.001$ ) on day 0 compared to all other time points. In addition, P3 progeny had greater ( $P < 0.001$ ) concentrations of Ig (A and G) on day 0, and greater ( $P = 0.041$ ) concentrations of IgG on day 14 compared to P1 progeny.

Denature gradient gel electrophoresis was used to evaluate the microbial fingerprint of dam and progeny feces. Diversity indices (Shannon's and Simpson's) represent the differences of the bacterial species within the microbial population while each index weighs species richness and evenness slightly differently. Shannon's index incorporates species richness (number of species, or in this case, PCR-DGGE bands) and evenness (the relative distribution of species) and Simpson's index takes into account the number of species present, as well as the relative abundance of each species. An increasing Shannon's index signifies a more diverse microbial population, while a decreasing Simpson's index indicates a greater diversity. Collectively, differences in similarity indicate the presence of different bands (i.e., bacterial species)

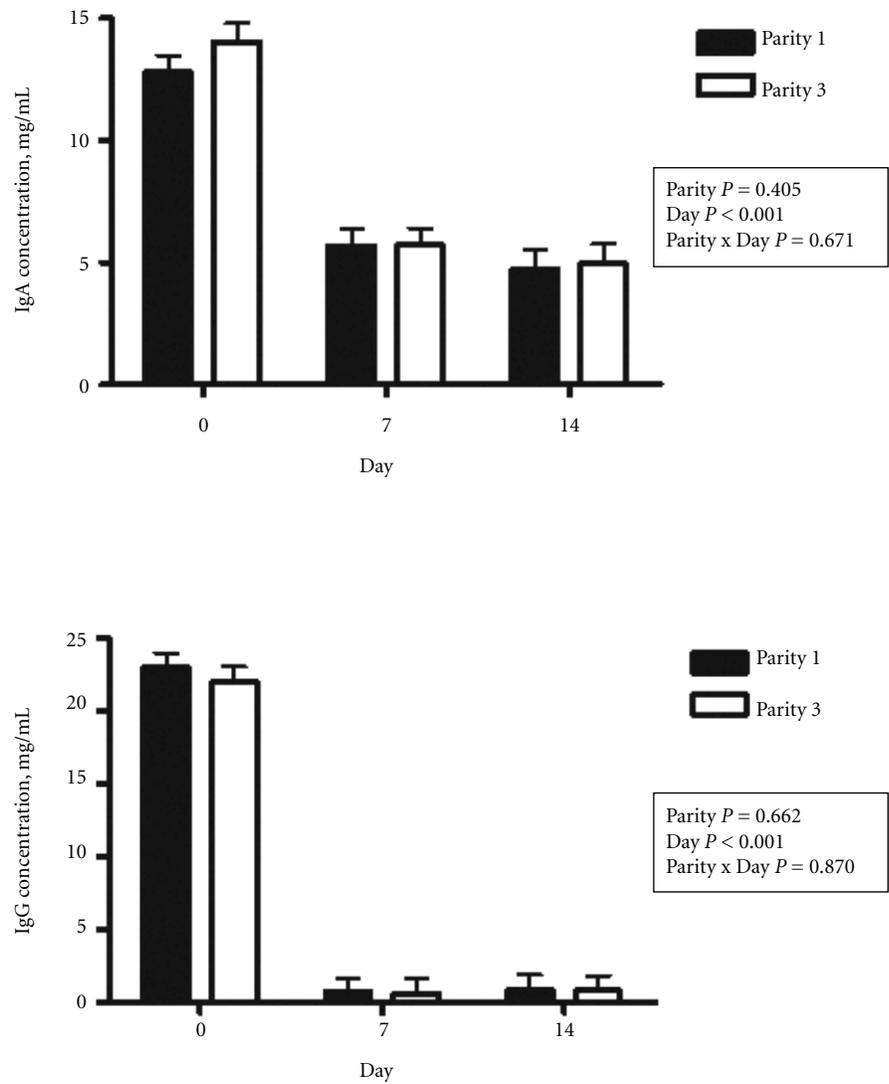
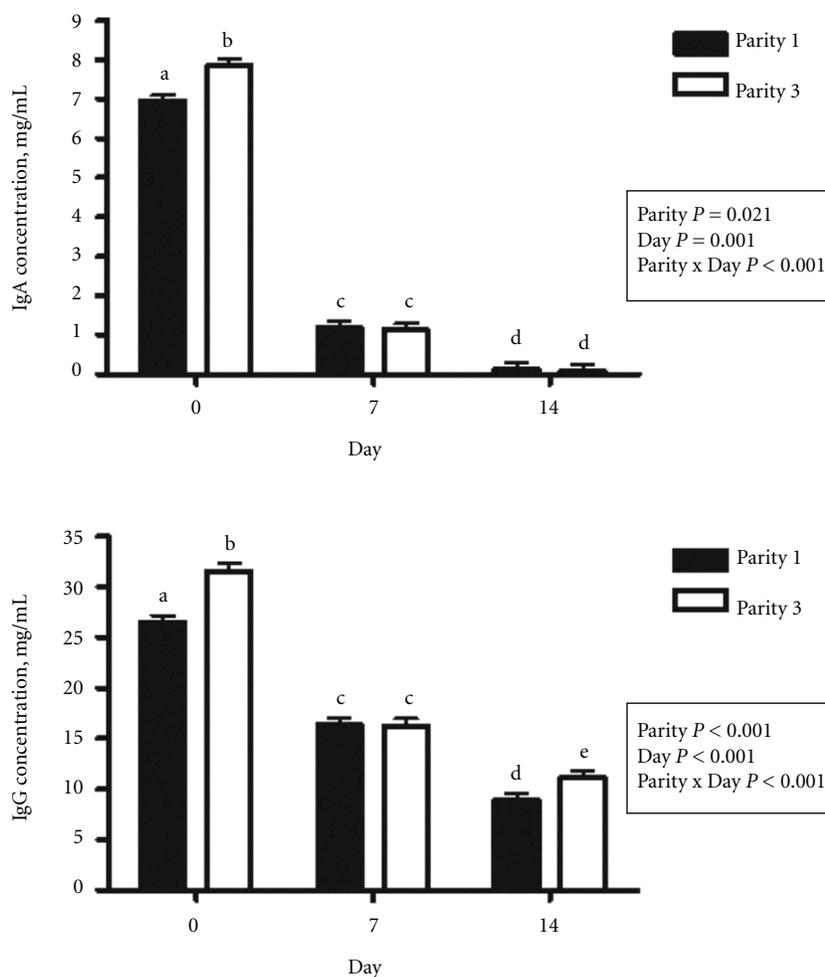


Figure 2. Concentrations of IgA (top panel) and IgG (bottom panel) in colostrum, mid-lactation (7 days following parturition; Mid-lac), and late lactation (14 days following parturition; Late-Lac) milk samples obtained from parity (P) 1 and 3 dams. Each bar represents the least squares mean ( $\pm$  SEM) of 56 and 49 observations for P1 and P3 dams, respectively.



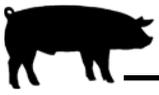
**Figure 3.** Circulating concentrations of IgA (top panel) and IgG (bottom panel) in serum obtained from the progeny of parity (P) 1 and 3 dams. Immunoglobulin concentrations were evaluated in serum obtained at 1, 7, and 14 days post-farrowing. Each bar represents the least squares mean ( $\pm$  SEM) of the progeny (6 pigs/litter) of 56 and 49 observations for P1 and P3 dams, respectively.

and differences in microbial diversity indicate an overall change in microbial community complexity. There were no effects of dam parity on fecal microbial fingerprinting (diversity or similarity indices) in samples obtained from dams or their progeny ( $P > 0.50$ ; data not shown).

### Conclusion

Litter performance and transfer of passive immunity may be affected by dam parity. The level of passive immunity acquired may directly affect the development of active immunity and indirectly affect the health and performance of the piglet. The results described in this report suggest that mature dams ( $\geq P3$ ) may provide their progeny with advantages in provision of passive immunity.

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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 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  $11 \pm .8$ . 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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