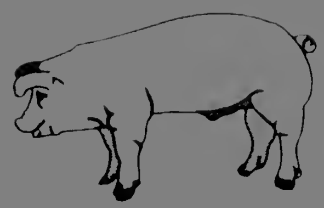
1998 UNIVERSITY OF ILLINOIS SWINE RESEARCH REPORTS



College of Agricultural, Consumer and Environmental Sciences

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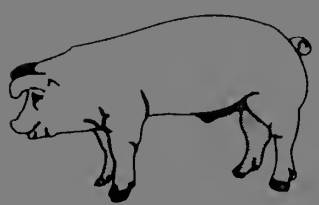

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Metric Conversions

Some of the results in this proceedings are reported in the English system (pounds, inches). However, all scientific meetings and journals require that data be presented in the metric (kilograms, meters, etc.). Therefore, some of the papers in this proceedings may be reported the metric system. Carefully observe the units to properly understand each paper. Following some of the conversion factors that may be useful to you in understanding metric measure

Metric		English
Length		
1 millimeter (mm)	=	0.03937 inch
1 centimeter (cm)	=	0.3937 inch
1 meter	=	39.37 inches
	=	3.281 feet
	=	1.094 yards
1 kilometer (km)	=	0.6214 mile
Area		
1 square centimeter (cm^2)	=	0.155 square inch
1 square meter (m^2)	=	1.196 square yards
	=	10.764 square feet
1 hectare (10,000 m^2)	=	2.471 acres
1 square kilometer (km^2)	=	0.386 square miles
	=	247.1 acres
Weight		
1 gram (g)	=	0.03527 ounce
1 kilogram (kg)	=	35.274 ounces
	=	2.205 pounds
1 metric ton (t)	=	0.984 ton (long)
	=	1.102 tons (short)
	=	2204.6 pounds
Volume per unit area		
1 liter/hectare (l/ha)	=	
Temperature Conversions		
Centigrade (Celsius)	=	
Fahrenheit	=	

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WWW Home Page-"PorkNet"

A new endeavor that the swine faculty in the Department of Animal Sciences and the Cooperative Extension Service Animal Systems Team has undertaken is the development of a "Swine" Home Page on the World Wide Web. It is referred to as **PorkNet**.

PorkNet is an integrated, information access, technology transfer system for the purpose of addressing the needs of the swine sector within Illinois. The program is led by the University of Illinois Department of Animal Sciences in collaboration withe the Departments of Agricultural and Consumer Economics (ACE) and Agricultural Engineering from within the College of Agricultural, Consumer and Environmental Sciences (ACES) and the College of Veterinary Medicine.

The goal of **PorkNet** is to provide the swine industry of Illinois with information in a rapid and timely manner to facilitate decision making. There will be two principle elements, focusing on information collection and dissemination, and the development of novel approaches to information delivery. This page is also designed to showcase the Department of Animal Sciences at the University of Illinois and the Animal Systems Team (Swine) along with faculty/staff from other departments within the College of Agricultural, Consumer and Environmental Sciences (ACES). The Department of Animal Sciences desires to tie the Illinois Pork Industry to the University of Illinois.

As an electronic communications and information system on the Internet, **PorkNet** takes advantage of the newest technologies to integrate up-to-date research information with data collected on individual operations to provide decision making tools for use by producers. **PorkNet** will also link other educational, association and industry related organizations to each other and to resources around the world. **PorkNet** will allow viewers to connect via electronic mail (e-mail), and provide an instantaneous method for asking questions and receiving answers from a panel of swine experts from a wide range of disciplines.

Each week, the **PorkNet** Web site will provide a **"Current Topic"**, i.e., the latest information or research findings from various discipline areas. An **"Ask an Expert"** section allows producers to send a question to experts in such areas as nutrition, genetics, reproductive physiology, buildings, ventilation, waste management, economics, veterinary medicine (swine health), business management, immunology, swine behavior and lactation biology. The question and answer exchange is shared on **PorkNet** for the benefit of all users.

The **PorkNet** design team is led by Dr. Mike Ellis, with assistance from Gilbert Hollis, Matthew Wheeler and Walt Hurley with the Department of Animal Sciences at the University of Illinois. Collaborating with them is Floyd Davenport and Chuck Kibler, Illinois Cooperative Extension Service Office of Computer Coordination.

The URL for PorkNet is: **www.aces.uiuc.edu/~pork**

Assessment of Multiple Concurrent Stressor Effects in Swine

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Summary

The effects of many single stressors have been reported, but how pigs perform when subjected to more than one or two stressors at a time, as is common in commercial swine production, has not. To study this, 256 Yorkshire × Hampshire or purebred Duroc pigs (34.7 ± 0.5 kg) were subjected to one of the eight treatment combinations (2 × 2 × 2 factorial) of ambient temperature [consant thermoneutral (24° C) or high cycling temperature (28-34° C)], stocking density (.56 m^2 or .25 m^2/pig) and social group (static group or regrouped at the start of wk 1 and 3) during a 4-wk experiment. The stress of high temperature, high stocking density and regrouping depressed 4-wk ADG by 12%, 16% and 10% ($P<.05$); and ADFI by 7%, 6% and 5%, respectively ($P<.05$). Out of a possible 60 stressor interactions for ADG, ADFI and G:F, there were no significant three-way interactions and only six two-way interactions, suggesting the effects of the individual stressors were additive (i.e., the stressors' effects on performance were similar regardless of whether they were imposed singly or in combination). Thus, when pigs were subjected to all three stressors simultaneously ADG, ADFI, and G:F were depressed by 31%, 15%, and 18%, respectively. Stressor additivity was further corroborated by examining the effect of "stressor order," or the number of stressors imposed simultaneously. As the number of stressors increased from 0 to 3, ADG, ADFI and G:F decreased linearly. These data suggest that multiple concurrent stressors affect growth performance of pigs in a predictable fashion (i.e., additively) and indicate that avoidance or removal of a given stressor is advantagous even when other uncontrollable stressors persist.

Introduction

The effects of many single stressors and a few two stressor complexes have been reported for swine. While the results indicate that stressors such as high ambient temperature (Close et al., 1978; Lopez et al., 1991; Nienaber et al., 1991; Xin and DeShazer, 1992), regrouping (Bjork et al., 1988; McGlone and Curtis, 1985), and restricted floor space (Kornegay and Notter, 1984; Kornegay et al., 1993a,b; NCR-89 Committee on Confinement Management of Swine, 1993), reduce feed intake and weight gain, it is difficult to extend information from single stressor studies to production settings because pigs usually experience several stressors at once. Recently, the effects of six concurrent stressors and effects of sequential stressors on performance and several physiological and pathological traits in the chick have been reported (McFarlane et al., 1989a,b,c; Johnson et al., 1991). When factorial combinations of six stressors

were imposed, effects generally were additive. For example, the percentage depression in feed intake and growth, respectively, increased linearly as the number of simultaneously imposed stressors increased. Whether the effects of multiple concurrent stressors in swine are additive, synergistic or antagonistic is not known. A better understanding of how several stressors acting together affect pigs' overall well-being and performance will enhance progress in management of animal environments.

Materials and Methods

Two hundred and fifty-six pigs (Yorkshire and Hampshire crossbreds and purebred Durocs) with an initial body weight of 34.7 ±.50 kg were used in two 4-wk trials. Within each trial, after taking into consideration body weight, gender and litter of origin, pigs were assigned to one of eight treatments in a 2^3 factorial arrangement. Treatments were imposed in two adjacent mechanically ventilated rooms. Two environmental temperatures (thermoneutral 24° C and hot diurnal temperature cycling from 28 to 34° C) were imposed in each room across two trials. The 2^2 factorial arrangement of space allocation (.56 and .25 m^2/pig) and regrouping (static group or regrouped) were imposed on a pen-within-room basis.

The two rooms were virtually identical, each having eight pens that were each equipped with a two-hole feeder, nipple waterer and a partially slotted floor. Both rooms were mechanically ventilated and had adjustable baffle inlets along one side that were adjusted to provide uniform air flow and velocity in both rooms. The air exchange rate was adjusted to be the same in both rooms to minimize differences in air quality. Aerial ammonia and hydrogen sulfide concentrations were measured at pig level at the center of each room during week 1 and 3 to ensure relatively equal air quality. Ammonia concentration in the thermoneutral and high ambient temperature rooms averaged 5 and 4 ppm, respectively. Hydrogen sulfide was not detected in either room. Relative humidity and temperature were monitored at pig level throughout the study using a hygrothermograph. A 24-h lighting regimen was employed in both rooms.

Pigs were provided *ad libitum* access to a corn-soybean meal-based diet that was formulated to exceed NCR (1988) nutrient requirements for grower pigs (17% CP, .9% lysine, and 3296 Kcal ME/kg). Eight pigs were assigned to each pen, and following a 1-wk acclimation period at .56 m^2/pig and 24° C, stressors were imposed. The room used for the hot diurnal environment was programmed to cycle from a low of 28° C from 2400 h to 0600 h to a high of 34° C from 0700 h to 2300 h. Pen size was adjusted to provide .25 m^2/pig for pigs assigned a treatment requiring restricted space allocation. Social stress was induced at the beginning of week 1 and 3 by regrouping pigs. This was accomplished as follows: Within each room for each experiment the 2^2 factorial arrangement of space allocation and regrouping was replicated twice. Four randomly selected pigs from one treatment replicate were switched with four randomly selected pigs from the other (i.e., pigs switched pens but not treatments). At the beginning of week 3, four pigs from one replicate were regrouped with the four unfamiliar pigs from the other, creating again, two treatment replicates with unfamiliar pigs.

Pig body weight and feed disappearance were determined at 1-wk intervals during the 4-wk

experiment so that ADG, ADFI, and G:F could be calculated. Each pen was video-recorded for 24 h during week 2 and 4. The duration of standing, lying, and sitting behavior was estimated by scan sampling every 15 min. At the end of each trial, blood samples were collected from 4 randomly selected pigs from each pen. Blood smears were made in duplicate from each sample and stained for differential leukocyte counts. Basal plasma cortisol was determined as was the cortisol response of pigs to exogenous ACTH after treatment with dexamethasone.

All data were analyzed with the PROC GLM procedures of SAS (1990). The statistical model included the main effects of trial, temperature, space allowance and regrouping, and all two- and three-way stressor interactions. Data were also categorized according to the number of stressors making up the stressor complex. Pigs were subjected to 0 (control), 1 (either high diurnal temperature, regrouping, or reduced space allocation), 2 (high diurnal temperature and regrouping; high diurnal temperature and reduced space allocation; and regrouping and reduced space allocation), or 3 stressors (high diurnal temperature and regrouping and reduced space allocation). Data were subjected to curvilinear regression analysis to detect whether there was deviation from a linear response (McFarlane et al., 1989).

Results

To study the effects of multiple concurrent stressors on growth performance, pigs were subjected to one of the eight treatment combinations (2 × 2 × 2) of environmental temperature (24° C or 28-34° C), space allowance (.56 m^2 or .25 m^2/pig), and social group (static group or regrouped) in a 4-wk experiment. The main effects of each stressor for ADG, ADFI and G:F for the entire trial and during weeks 1, 2, 3 and 4 are shown in Table 1. Exposure to high cycling temperature, reduced space allowance, or regrouping, depressed ADG over the 4-wk experiment by 11.9%, 16.4%, and 9.6%, respectively ($P<.05$). Stressors also depressed feed intake: Average daily feed intake for pigs subjected to high cycling temperature, reduced space allowance, or regrouping was depressed by 7.4%, 6.0%, and 5.0%, respectively ($P<.05$; Table 1). Whereas temperature and regrouping did not influence 4-wk G:F ($P>.05$), reduced space allowance depressed feed conversion efficiency by more than 10%. The depression in growth, feed intake and feed efficiency by stressors varied from one week to the next. For instance, reduced space allowance and high cycling temperature depressed ADG and ADFI more during week 4 than during week 1 (Table 1). In fact, growth performance was not affected by temperature until week 3 and 4.

Of the possible 60 interactions, only 6 were significant. A temperature × regrouping interaction was detected for ADG during week 1, 3 and 4, and for G:F during week 1 ($P<.05$). During week 1 and 3, the effect of regrouping was greater in the 24° C thermoneutral environment than in the high cycling temperature. The opposite was true during week 4, however. A space allowance × regrouping interaction was detected for ADG during week 2 and over the entire 4-wk experiment ($P<.05$). The interaction indicated that the effect of regrouping was less when space allowance was restricted.

The 4-wk ADG, ADFI and G:F for pigs on each of the eight treatment combinations are presented in Table 2. The depression of ADG, ADFI, and G:F caused by imposing each stressor

individually was similar (P>.05). In addition, there were no differences in the ADG, ADFI, and G:F for pigs subjected to the different two-stressor complexes. Overall, when pigs were subjected to the three stressors simultaneously, ADG, ADFI, and G:F were depressed by 30.8%, 15.1%, and 17.5%, respectively. The treatments were also categorized according to the number of stressors they comprised (see Table 2). Regression analysis detected a linear and negative effect of the number of stressors imposed on ADG, ADFI, and G:F (Figure 1).

No behavioral or physiological parameter examined proved to be particularly useful for predicting the effects of stress on growth performance of pigs with the possible exception of the neutrophil:lymphocyte ratio. Regression analysis detected a linear and positive effect of the number of stressors imposed on the neutrophil:lymphocyte ratio, but the model explained only 7% of the treatment variation. The packed cell volume, basal plasma cortisol concentration, and plasma cortisol after injection of dexamethasone and ACTH were not different between pigs subjected to the different stressors.

Discussion

To understand how different stressors that are present simultaneously influence growth performance, pigs were subjected to a factorial arrangement of treatments involving three stressors. The resultant data confirm previous studies which show that when presented alone, high ambient temperature, reduced space allowance, and regrouping depress growth, feed intake, and feed conversion efficiency. The important finding was that when these stressors were presented together, as is often the case in typical swine production systems, the effects were in general, additive. Because the effects were additive, these data indicate that removal of a single stressor can have substantial beneficial effects on the growth performance of pigs, even when a number of other uncontrollable stressors persist. They further suggest that it may be possible to predict the effects of multiple simultaneous stressors on growth performance of pigs if the effects of the individual stressors are known.

Because the underlying goal of this study was to determine if the effects of multiple stressors on growth performance of pigs were additive, synergistic or antagonistic, it was important that each individual stressor induce a significant depression in growth performance. Therefore, the type of stressor and the level of stress were chosen judiciously in order to ensure a detrimental effect on performance, but to still be within limits of what may be observed in typical production systems. Accordingly, the main effects of high ambient temperature, reduced space allowance and regrouping significantly depressed ADG and ADFI. These findings were predictable and are in agreement with a number of previous studies that reported the effects of similar stressors on growth performance of pigs.

That the three stressors were found to have significant negative effects on growth performance suggested that by imposing them simultaneously in the various factorial combinations one could effectively evaluate how different stressors interacted. Surprisingly, out of a possible 60 stressor interactions for ADG, ADFI and G:F, there were no three-way interactions and only 6 two-way interactions. In most cases, whereas in one week the interaction was antagonistic, in another week it was synergistic. The net effect was that for the performance over the 4-wk experiment, only one significant interaction was detected. A space allowance × regrouping interaction was

detected for ADG indicating the effect of regrouping was less when space allowance was restricted. That reduced space allowance increases the frequency of challenges to a pigs' personal space and, therefore, results in an increase in agonistic encounters, regrouping pigs that had reduced space allowance may not have been as stressful (i.e., perhaps pigs kept under reduced space allowance were acclimated to a relatively high level of agonistic behavior). Thus, if reduced space allowance and regrouping impinged upon the pig via a similar path (i.e., they both caused psychological stress and increased agonistic behavior), this may explain the antagonistic interaction. It should be noted, however, that other stressors which impinge upon the pig via a common path may exacerbate one another as is the case of aerial ammonia reducing the ability of pigs to clear bacteria from their lungs (Drummond et al., 1978) and increasing the nasal-turbinate shrinkage in pigs infected with *Bordetella bronchiseptica* (Drummond et al., 1981). Nonetheless, in the present study the effects of stressors were by-and-large, additive.

To further examine the additivity of stressors, treatments were categorized according to the number of stressors they comprised. This ranged from 0 for control to 3 for the treatment comprising all of the stressors. These data were subjected to curvilinear regression analysis to determine if increasing the number of stressors from 0 to 3 decreased performance in a linear or quadratic fashion. McFarlane et al. (1989b) used this approach to study the effects of six concurrent stressors on chick performance. They found that as the number of stressors increased, performance decreased linearly. When chicks were subjected to six stressors simultaneously, their growth rate over a 7-d trial was decreased by more than 60%. These authors also reported very few stressor interactions. The results of the current study are remarkably consistent with the results reported by McFarlane et al. (1989b) for chicks. For instance, there was a linear and negative relationship between the number of stressors imposed and ADG, ADFI and G:F. When pigs were subjected to three stressors simultaneously, ADG, ADFI, and G:F were depressed by 30.8%, 15.1%, and 17.5%, respectively. The regression equation for ADG indicates that the addition of each stressor resulted in an additional 10% depression in growth. It is important to point out, however, that not every stressor contributes equally as this may suggest. Moreover, it is not the mere presence or absence of a stressor that is important, but also the level of stress induced. For instance, pigs of this size may experience heat stress if kept at either 34° C or 30° C, but clearly the depression in growth would be greater at 34° C. Nonetheless, the present data suggest that in some cases the effects of multiple concurrent stressors on growth performance of pigs is equal to the sum of the stressors' individual effects.

Implications

Animal environments are complex and frequently comprise several stressors simultaneously. Despite this, whether stressor effects on growth performance are additive, antagonistic or synergistic is not known. This information is needed to better manage animals' environments. These data show that the detrimental effects of high ambient temperature, regrouping and reduced space allowance are, in general, additive. Therefore, removing a single stressor from a complex environment containing multiple stressors may substantially improve growth performance of pigs. This is important because some stressors are uncontrollable and therefore may be present even under optimal management. Furthermore, that the multiple concurrent

stressors affected performance in a predictable manner (i.e., additively), it may be possible to estimate the growth response of pigs in a complex environment comprising multiple stressors if the effects of the individual stressors are known.

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Table 1. Main effects of stressors on pig performance[1].

Item	Temperature (°C)		Space allowance (m^2)		Mixing		Av.SE
	24	28-34	.56	.25	Static	Mixed	
Week 1							
ADG, g [2]	676	704	752[a]	629[b]	721	660	24.2
ADFI, kg	1.75	1.71	1.77	1.69	1.77	1.69	.045
Gain:feed [2]	.39	.42	.43[a]	.37[b]	.41	.39	.015
Week 2							
ADG, g	773	707	799[a]	680[b]	804[a]	677[b]	24.4
ADFI, kg	1.88	1.86	1.91	1.83	1.93	1.81	.051
Gain:feed	.42	.38	.42	.37	.42	.38	.024
Week 3							
ADG, g	820[a]	596[b]	748[a]	669[b]	747[a]	669[b]	22.7
ADFI, kg	2.18[a]	1.87[b]	2.10	1.94	2.06	1.99	.066
Gain:feed	.38[a]	.32[b]	.36	.35	.36	.34	.017
Week 4							
ADG, g	772[a]	676[b]	822[a]	625[b]	760	708	25.1
ADFI, kg	2.27[a]	2.03[b]	2.23	2.06	2.21	2.08	.062
Gain:feed	.34	.33	.36[a]	.31[b]	.33	.34	.016
Weeks 1 to 4							
ADG, g	761[a]	670[b]	780[a]	652[b]	752[a]	680[b]	110
ADFI, kg	2.02[a]	1.87[b]	2.00[a]	1.88[b]	1.99[a]	1.89[b]	.029
Gain:feed	.38	.36	.39[a]	.35[b]	.37	.36	.007

[1] : Means within a row with different superscripts differ under each main effect ($P < .05$).
[2] : Temperature × mixing interaction ($P < .05$).

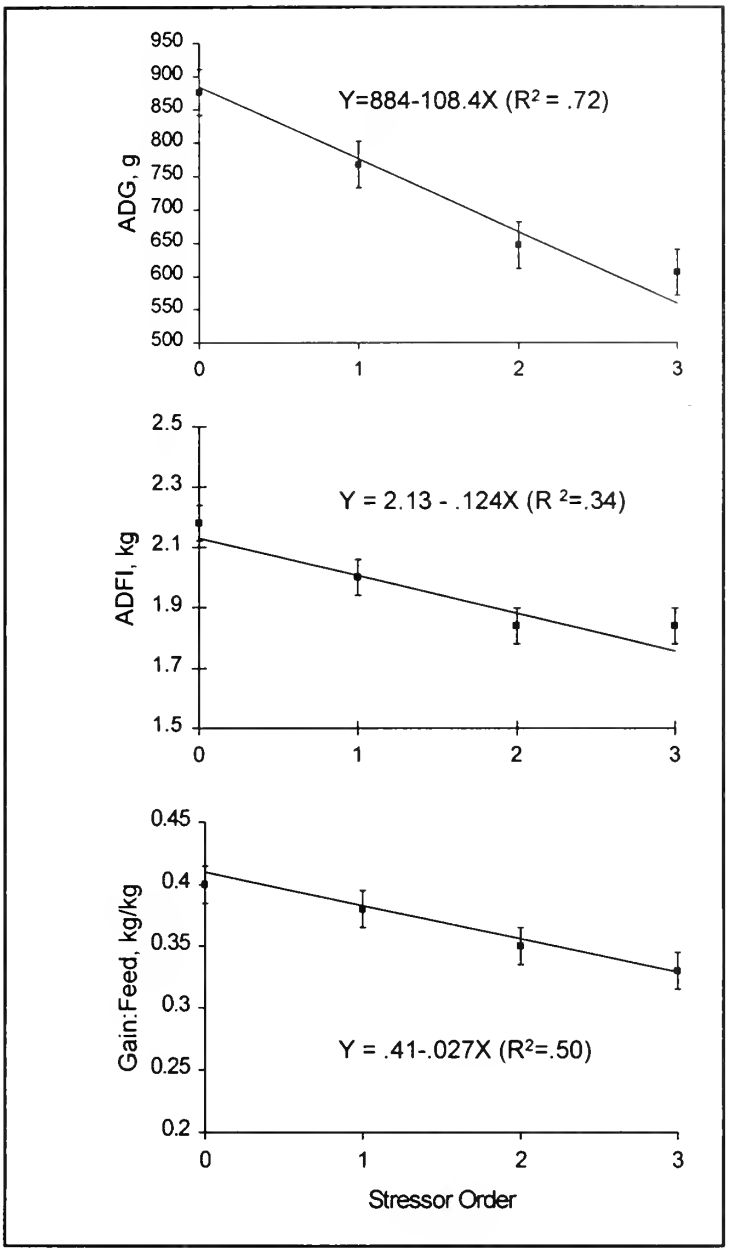
Table 2. Treatment effects on pig performance over the 4-wk experiment.

Treatment[1]			Performance[2]		
High Temperature	Restricted space allowance	Mixing	ADG	ADFI	Gain:Feed
			Mean	Mean	Mean
-	-	-	876^{a}	2.18^{a}	.40^{a}
+	-	-	792^{ab}	1.99^{bc}	.40^{a}
-	+	-	734^{bc}	2.00^{bc}	.37^{abc}
-	-	+	777^{ab}	2.01^{ab}	.39^{ab}
+	+	-	608^{d}	1.80^{d}	.34^{c}
+	-	+	676^{cd}	1.83^{cd}	.37^{abc}
-	+	+	657^{cd}	1.88^{bcd}	.35^{bc}
+	+	+	606^{d}	1.85^{bcd}	.33^{c}
Avg. SE			34.8	.059	.015

[1] - : No stress was applied.; +: Stress applied.

[2] Means within a column with different superscripts differ ($P < .05$)

Figure 1. Effect of stressor order on 4-week growth performance of pigs. The eight treatments were categorized according to the number of stressors they comprised (0, 1, 2 or 3) and data were subjected to curvilinear regression analysis. Results indicate increased stressor order decreased growth performance linearly and negatively.



Biochemical Markers of Sickness

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Department of Animal Sciences
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The emerging view is that sickness in pigs, manifesting as reduced feed intake and lowered lean muscle growth, may result from increased biosynthesis of certain cytokines. University of Illinois scientists (Webel et al., 1997) employed 72 pigs weighing 12 kg to test this hypothesis. The pigs were fasted for 12 hours, and then were given an i.p. injection of 5 μg/kg BW *E. Coli* lipopolysaccharide (LPS) to induce sickness. Blood samples were obtained at 0, 2, 4, 8, 12 and 24 hours post-injection while the pigs continued to fast. At 2 hours post-injection, plasma tumor necrosis factor-α (TNF-α) was elevated 10-fold. At 4 hours post-injection, plasma cortisol was elevated 6-fold and plasma interleukin 6 (IL-6) was elevated 200-fold. By 12 hours post-injection, all of these metabolites had returned to normal baseline values. However, beginning 2 hours post-injection, plasma urea nitrogen began increasing, and reached a peak (3-fold elevation) at 12 hours post-injection. Plasma levels of nonesterified fatty acids, triglycerides, glucose and α-1 acidglycoprotein (an acute-phase protein) were not affected by the LPS injection.

It is likely that macrophage-derived cytokines such as IL-6 and TNF-α are synthesized early in the course of an infection. This is accompanied by increased adrenal release of cortisol, probably because IL-6 and other proinflammatory cytokines stimulate neurons in the hypothalamus to secrete corticotropin-releasing hormone, which in turn causes pituitary secretion of ACTH. This cascade of events causes skeletal muscle degradation, which causes increased synthesis of ammonia, the nitrogenous precursor of urea. A striking finding was the marked elevation in plasma urea, which reached its peak 8 hours after peak levels of IL-6, TNF-α, and cortisol had been achieved.

Sickness and Amino Acid Requirements

Work in our laboratory with pigs and chicks has shown clearly that LPS-induced reductions in voluntary feed intake do not increase the dietary lysine requirement expressed in terms of concentration (i.e., % of diet or % of calories). In fact, the lysine requirement expressed in terms of daily intake to maximize feed efficiency and protein accretion has been found to decrease significantly.

The recent work of Williams et al. (1997a,b,c) evaluated lysine levels for pigs housed in facilities that either maximized or minimized exposure to disease and environmental antigens. The healthy pigs in all cases showed greater capacities for lean protein accretion than was the case for the unhealthy pigs. Also, the unhealthy pigs required lower levels of dietary lysine than the healthy pigs, resulting in substantially lower daily lysine requirements (g/day). These Iowa State University studies demonstrated that the efficiency of lysine utilization for protein accretion was not reduced in the unhealthy pigs. Instead, the capacity for protein accretion was reduced, and this agrees with our University of Illinois chick studies involving LPS dosing and its effects on lysine and threonine utilization (Webel et al., 1998).

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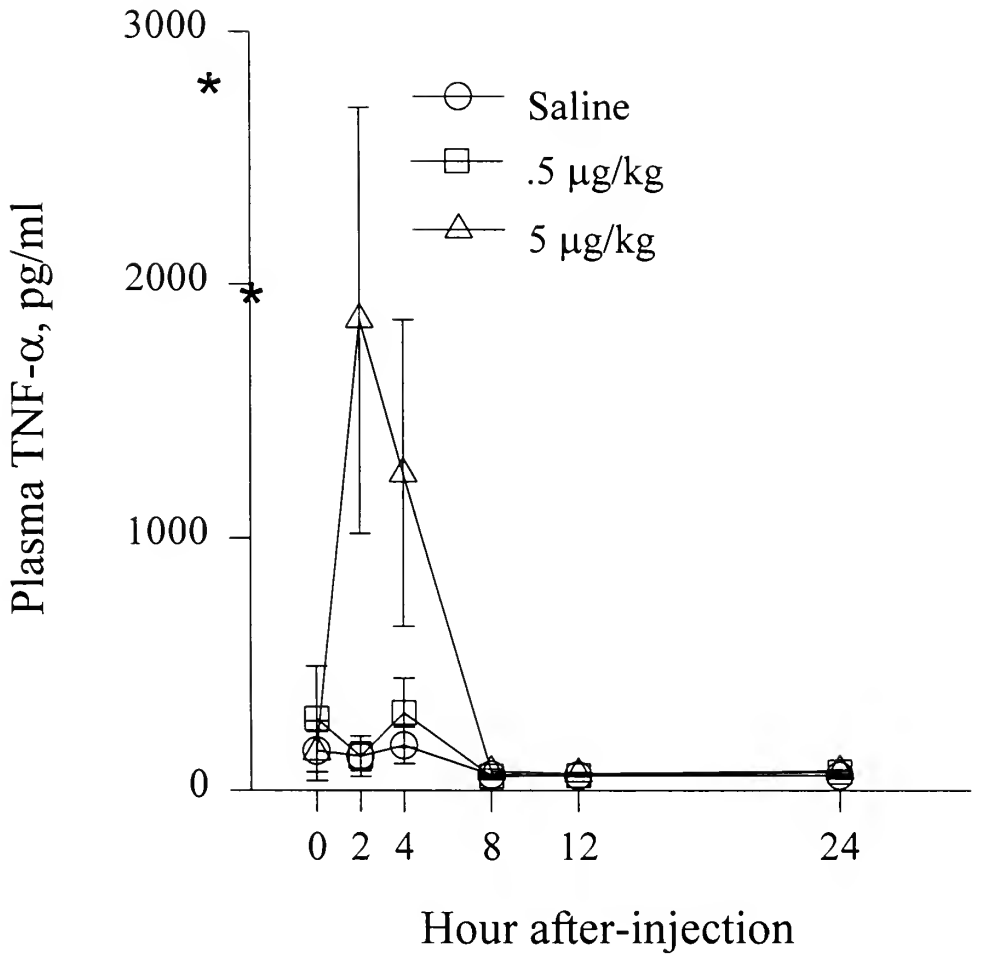

Figure 1. Plasma TNFα of pigs following a challenge dose of lipopolysaccharide (LPS). Pigs were injected i.p. with saline, .5 or 5µg/kg BW of LPS at 0 h. Feed was removed 12 h prior to injection and was not available throughout the 24-h period following injection. Asterisks indicate that a treatment mean at a given time period is different from the saline-injected control ($p < .05$).

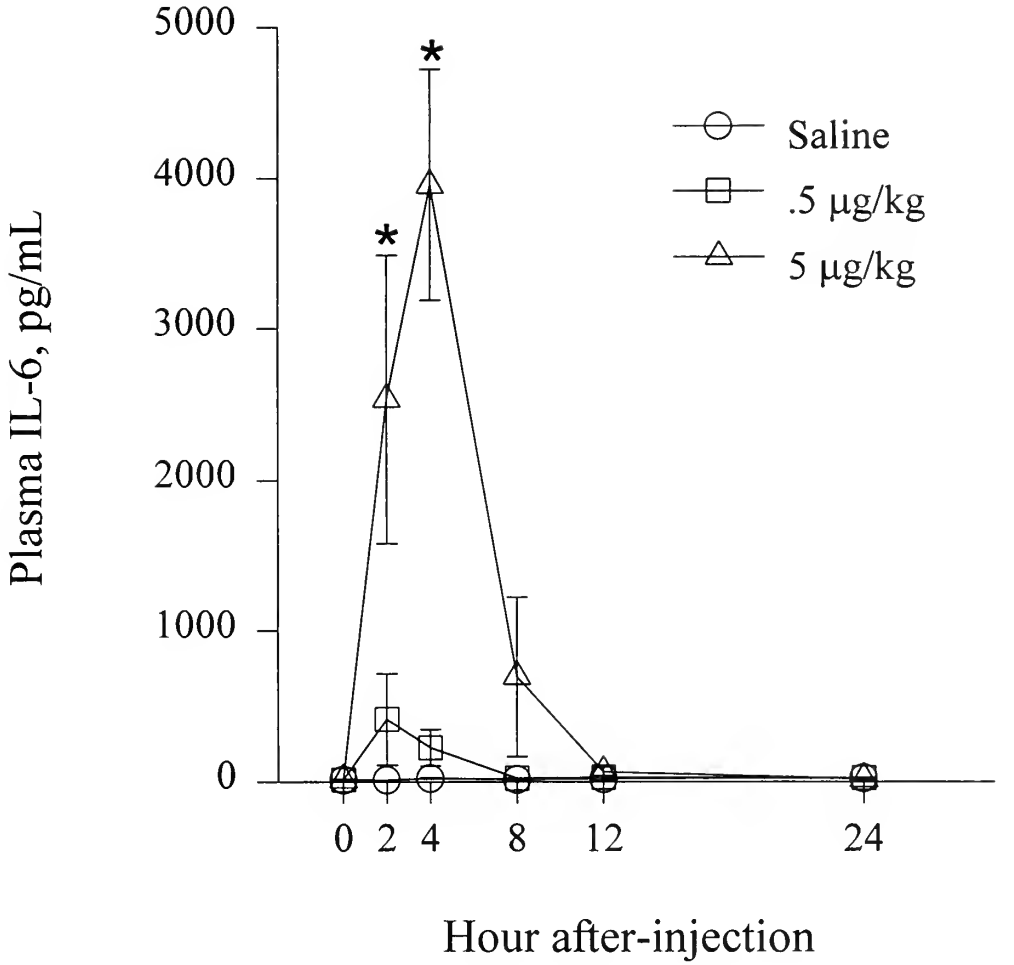

Figure 2. Plasma IL-6 of pigs following a challenge dose of lipopolysaccharide (LPS). Pigs were injected i.p. with saline, .5 or 5µg/kg BW of LPS at 0 h. Feed was removed 12 h prior to injection and was not available throughout the 24-h period following injection. Asterisks indicate that a treatment mean at a given time period is different from the saline-injected control ($p < .05$).

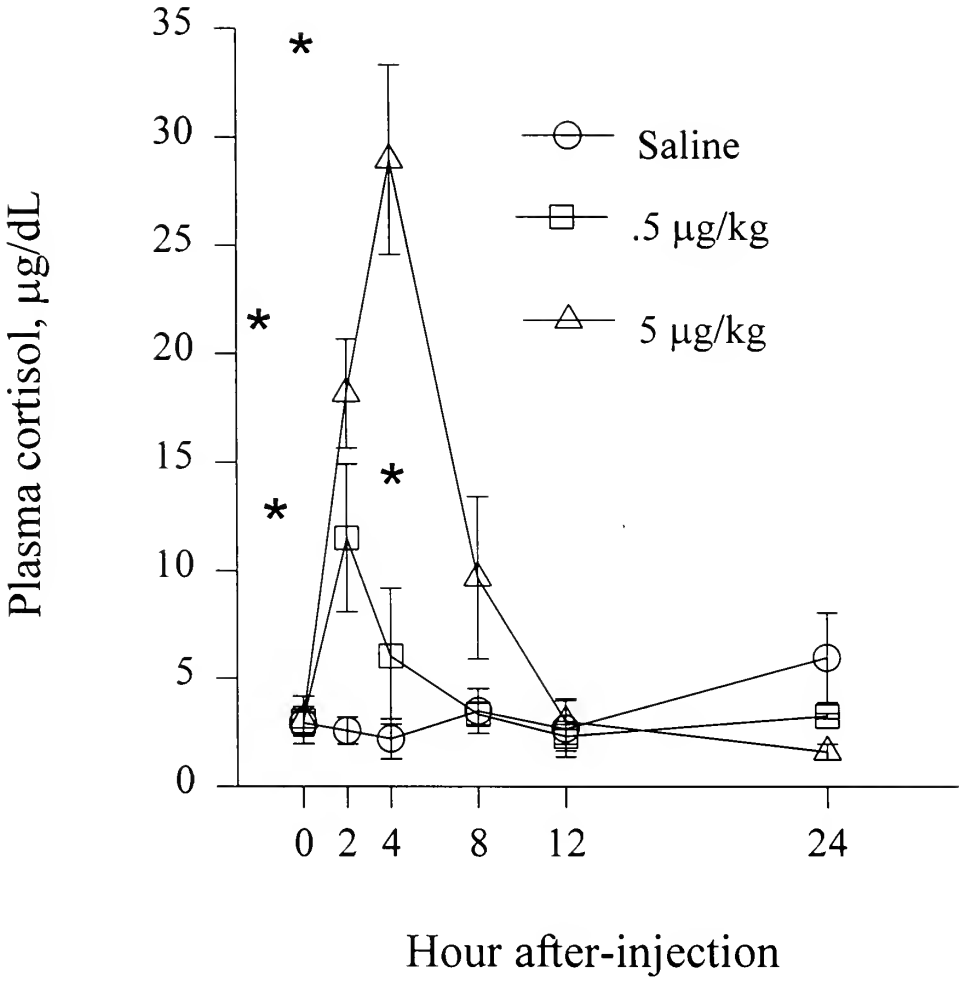

Figure 3. Plasma cortisol of pigs following a challenge dose of lipopolysaccharide (LPS). Pigs were injected i.p. with saline, .5 or 5µg/kg BW of LPS at 0 h.Feed was removed 12 h prior to injection and was not available throughout the 24-h period following injection. Asterisks indicate that a treatment mean at a given time period is different from the saline-injected control ($p < .05$).

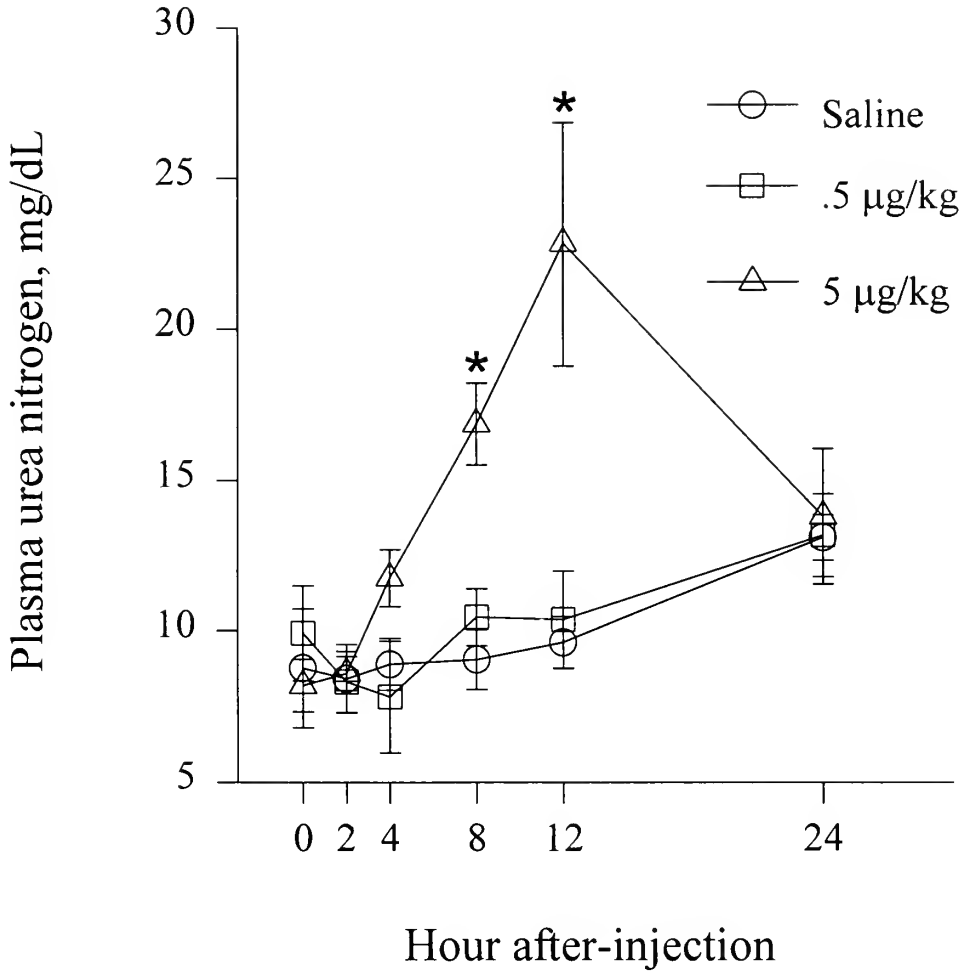

Figure 4. Plasma urea nitrogen of pigs following a challenge dose of lipopolysaccharide (LPS). Pigs were injected i.p. with saline, .5 or 5µg/kg BW of LPS at 0 h. Feed was removed 12 h prior to injection and was not available throughout the 24-h period following injection. Asterisks indicate that a treatment mean at a given time period is different from the saline-injected control ($p < .05$).

Activation of *in vivo* Matured Porcine Oocytes

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Introduction

Transgenic animals are produced by inserting 'foreign' DNA into one cell embryos. The foreign DNA is then able to integrate into chromosomes and subsequently be expressed in all somatic tissues of the resultant individual. This phenomena was initially demonstrated by microinjection of genes into the pronucleus of fertilized mouse ova. Transgenic technology could be very useful for introducing genes into domestic animals and in so doing, have great impact toward improving efficiency of animal agriculture. An excellent example of this in the swine industry is the introduction of growth hormone genes into pigs to accelerate their rate of growth. Unfortunately, microinjection into ova has greatly reduced survival of the embryos. In swine, the survival rate of the embryos was approximately 25 to 35%. Efficiency of producing offspring carrying foreign genes (transgenic) by this technique ranges from 0.04 to 1.7% (Rexroad and Pursel, 1988). However, if the embryo survives, the percentage of offspring expressing the foreign genes or 'transgenes' is much higher, about 17 to 100% in swine. It is therefore important to develop highly efficient methods to introduce genes into swine.

One alternative method, previously developed in mice (Evans and Kaufman, 1981), may be very useful in increasing the efficiency of producing transgenic swine. This strategy involves the injection of embryonic cells into embryos to produce 'hybrid' embryos composed of two or more distinct cell lines. These embryos are called chimeras. Embryonal stem cell lines (ES-cells) which are derived from embryos must be able to produce all tissues of an individual. Once isolated, ES-cells may be grown *in vitro* for many generations producing an unlimited number of identical cells capable of developing into fully formed adult chimeras. These cells may then be transformed genetically before being used to produce chimeric embryos (Bradley et al., 1984). When these transformed cells form the gonads and participate in the formation of sperm and eggs, the offspring that are produced by these chimeric individuals will be transgenic. Chimeric individuals can act as 'founder' stock to produce many individuals that carry the desirable gene(s).

Recent developments (Wilmut et al., 1997) in nuclear transfer (NT) or cloning technology could provide methods which could directly allow the generation of entire offspring from a single, genetically transformed or transgenic ES cell. This method might allow us to bypass the production and selective breeding of chimeric individuals by producing germ-line transgenics directly from individual ES cell nuclei. In addition, it could provide for the production of large numbers of genetically identical transgenic pigs which provide defined genotypes that are necessary for some applications of this technology, i.e. selection for disease resistance, uniformity in production traits and reproductive performance.

Unfortunately, reported attempts to "clone" pigs have resulted in only one piglet which was produced by nuclear transfer of a single 4-cell blastomere into an enucleated oocyte (Prather et al., 1989). Although this one success has demonstrated that many of the techniques used to manipulate the embryo and the transferred nucleus can be successful, the production of nuclear transfer piglets has not been repeated, partly due to accumulated low efficiencies throughout the multiple step process. The development of embryonic stem cells in pigs could provide the opportunity to successfully clone pig embryos, but the parameters required for nuclear transfer with these cells remain to be determined.

One of the major problems in the production of piglets from nuclear transfer is the development of

methods for successful and reliable activation of the cytoplasm (the non-nucleus portion) of the recipient oocyte. This is the portion that will receive the transferred nucleus from the embryonic stem cell in order to produce NT embryos. It was recently (Campbell et al. 1993, 1994) reported a method, in sheep, to obtain cytoplasm acceptable for NT with nuclei from any stage of the cell cycle (The Universal Recipient). Although numerous treatments have been attempted to activate porcine oocytes, electrical activation has been the most popular method. However, the majority of reports have utilized *in vitro* matured (IVM) porcine oocytes which may not be comparable to *in vivo* matured oocytes.

The objectives of these studies were to determine the effects of different treatments (previously shown to be effective in other species) on activation of *in vivo* matured porcine oocytes. These treatments were: 1) ethanol; 2) cold shock; 3) sham enucleation; 4) electroactivation; 5) electroactivation and culture with cycloheximide; and 6) electrical field strength.

Rationale

Traditional methods to produce cloned animals utilize early cleavage staged blastomeres transferred to enucleated oocytes. The oocytes are then fused with the transferred nucleus, induced to resume cytoplasmic and nuclear development and cultured or transferred to surrogate mothers. The recent development of embryonic stem cell lines in the pig provides a source of small cells of potentially unlimited number for nuclear transfer. Because of the novelty of these cells, no information is available regarding appropriate methods for each of the multiple steps of nuclear transfer. The following experiments were designed to optimize the production of clonal offspring from transfer of embryonic stem cells in the pig.

Materials and Methods

In all four experiments, gilts were observed for estrus every 12 hours. In Experiment 1, oocytes from one crossbred Pietran x Meishan and three crossbred Duroc x Meishan gilts were collected. In Experiment 2, oocytes from three Duroc and three crossbred Duroc x Meishan gilts were collected. Upon onset of estrus, gilts in the first two experiments were given 500 IU of human chorionic gonadotropin (hCG) to promote ovulation 40 to 44 hours following hCG injection. In Experiment 3, oocytes from one Meishan and two crossbred Duroc x Yorkshire gilts were collected. In Experiment 4, oocytes from four Meishan, two Yorkshire and five crossbred Duroc x Yorkshire gilts were collected.

In Experiment 1, oocytes (n = 49) were surgically flushed with Beltsville embryo culture medium (BECM; Dobrinsky et al., 1996) approximately 40 to 44 hours after hCG injection. Ethanol treatment (four reps) was performed by placing oocytes in BECM with 7% ethanol for 5 minutes (n = 25) whereas control oocytes were placed in BECM without ethanol for 5 minutes (n = 24). Oocytes were washed three times with BECM and modified Whitten's medium + 1.5% bovine serum albumin (BSA; Beckmann and Day, 1993) and placed into 50 microliter drops of modified Whitten's medium + 1.5% BSA under paraffin oil. Oocytes were cultured for 20 hours at 39°C in a humidified 5% CO_2 in air environment. Following the culture period, oocytes were stained with Hoechst 33342 (2.5 micrograms/milliliter; Sigma, St. Louis, MO) for 15 minutes, washed, viewed under light microscropy and examined under fluorescence for the presence of a second polar body and/or a pronucleus.

In Experiment 2, oocytes (n = 66) were surgically collected approximately 40 to 44 hours after hCG injection from oviducts with BECM. Oocytes were washed three times with BECM and modified Whitten's medium + 1.5% BSA and placed into 50 microliter drops of modified Whitten's medium + 1.5% BSA under paraffin oil. Cold shock treatment (six reps) was performed by culturing oocytes for 20 hours at 25°C in 5% CO_2 in air (n = 34) whereas control

oocytes were cultured at 39°C in 5% CO_2 in air (n = 32). At cessation of the culture period, oocytes were stained with Hoechst 33342 (2.5 micrograms/milliliter) for 15 minutes, washed, viewed under light microscropy and examined under fluorescence for the presence of a second polar body and/or a pronucleus as well as two-cell parthenote formation.

In Experiment 3, oocytes (n = 36) were surgically flushed from crossbred Duroc x Yorkshire gilts whereas the Meishan gilt was euthanized and oocytes were flushed from oviducts with BECM approximately 40 to 44 hours after the onset of estrus. Sham enucleated oocytes were placed in micromanipulation medium (BECM + 7.5 micrograms/milliliter cytochalasin B) and a small volume of cytoplasm was removed opposite the first polar body with a Nikon diaphot microscope (Nikon Inc., Melville, NY) equipped with Narishige micromanipulators (Narishige Co., Ltd., Tokyo, Japan). Control oocytes were placed in micromanipulation medium but not micromanipulated. Oocytes from both treatments were washed three times in BECM and modified Whitten's medium + 1.5% BSA and placed in 50 microliter drops of modified Whitten's medium + 1.5% BSA under paraffin oil. Oocytes were cultured for 20 hours at 39°C in a humidified 5% CO_2 in air environment. After the culture period, oocytes were stained with Hoechst 33342 (2.5 micrograms/milliliter) for 15 minutes, washed, viewed under light microscropy and examined under fluorescence for the presence of a second polar body and/or a pronucleus.

Approximately 40 to 44 hours after the onset of estrus, *in vivo* matured oocytes (n = 156) in Experiment 4 were surgically collected from Yorkshire and crossbred Duroc x Yorkshire gilts whereas Meishan gilts were euthanized and oocytes were flushed from oviducts with BECM. Oocytes were randomized to three treatments. The treatments were: 1) culture (CNTRL), 2) electroactivation and culture (ELECTRO), and 3) electroactivation and culture in presence of cycloheximide (CYCLO). Oocytes from the ELECTRO and CYCLO treatments were placed in .3 *M* mannitol (pH = 7.2) to equilibrate. Next, they were placed between two wire electrodes 1-millimeter apart (microslide 450; BTX Inc., San Diego, CA) in .3 *M* mannitol. A 30 microsecond DC pulse was given with a BTX Electro Cell Manipulator 200 (BTX Inc., San Diego, CA). Initially, a field strength of 2.8 kilovolts/centimeter was used and a second study was performed with a field strength of 1.3 kilovolts/centimeter. Oocytes from CNTRL and ELECTRO treatments were washed three times with BECM and modified Whitten's medium + 1.5% BSA and placed into culture with modified Whitten's medium + 1.5% BSA under paraffin oil. Oocytes from the CYCLO treatment were washed three times with BECM and modified Whitten's medium + 1.5% BSA + cycloheximide (5 micrograms/milliliter; Sigma Chemical Co., St. Louis, MO) and placed into culture with modified Whitten's medium + 1.5% BSA + cycloheximide (5 micrograms/milliliter) under paraffin oil. Within all treatments, oocytes were cultured for 24 hours at 39°C in a humidified 5% CO_2 in air environment. Following culture, oocytes were washed three times with BECM, placed in .075 *M* KCl for 10 minutes and placed in 10% formalin overnight. Next, oocytes were mounted on glass slides, placed in acetic acid:ethanol (1:2) for 48-72 hours and stained for 2 minutes with 2% aceto-orcein. Oocytes were immediately examined under light microscropy for the presence of pronuclei and the formation of two-cell parthenotes.

In Experiment 1, 2 and 3, the effect of treatment on oocyte activation and two-cell parthenote formation was examined by Chi-square analysis. In Experiment 4, the effects of treatment and pulse strength on oocyte activation and two-cell parthenote formation were examined by Chi-square analysis.

Results

Results from Experiment 1 indicated no difference between control and ethanol activated oocytes (Figure 1). Activation rates for oocytes from both treatments were 8%. In Experiment 2, however, *in vivo* matured oocytes that were cultured in modified Whitten's medium + 1.5% BSA at 25°C (cold shock treatment) activated at higher rates than control oocytes cultured at 39°C for 20

hours (Figure 2). Rates of activation were 74% for cold shock oocytes and 50% for control oocytes. Both treatments produced a high percentage of two-cell parthenotes (47% for cold shock and 37% for control oocytes; Figure 3). The sham enucleation treatment in Experiment 3 activated oocytes at higher rates than control oocytes (Figure 4). Activation rates for sham enucleated and control oocytes were 62 and 27%, respectively.

In Experiment 4, differences in pronuclear formation were detected among all treatments (Figure 5). Since no differences were detected between 1.3 kilovolts/centimeter and 2.8 kilovolts/centimeter field strengths, these data were combined. Activation rates were highest for CYCLO oocytes (76%), intermediate for ELECTRO oocytes (49%) and lowest for CNTRL oocytes (13%). Rates of parthenogenetic two-cell formation (Figure 6) were highest for the ELECTRO treatment (33%), intermediate for the CNTRL treatment (7%) and lowest for the CYCLO treatment (0%). Within the ELECTRO treatment, a field strength of 2.8 kilovolts/centimeter promoted higher rates of two-cell parthenote formation than a field strength of 1.3 kilovolts/centimeter, however within the CYCLO treatment no two-cell parthenotes formed for either field strength.

Conclusions

Results from Experiment 1 indicated that ethanol treatment of *in vivo* matured porcine oocytes was unsuccessful in improving activation rates following 20 hours of culture. Experiment 2 demonstrated that culture of *in vivo* matured oocytes at 25°C resulted in higher activation rates than culture at 39°C for 20 hours. Following 20 hours of culture in Experiment 3, sham enucleated oocytes activated at higher rates than control oocytes. In Experiment 4, activation rates were highest for the CYCLO treatnent, the ELECTRO treatement was intermediate and the CNTRL treatment was lowest following 24 hours of culture. Rates of parthenogenetic two-cell formation were highest for the ELECTRO treatment, intermediate for the CNTRL treatment and lowest for the CYCLO treatment. Field strength had no significant effect on activation rates for either treatment but did influence two-cell parthenote formation. Within the ELECTRO treatment, a field strength of 2.8 kilovolts/centimeter promoted higher rates of two-cell parthenote formation than a field strength of 1.3 kilovolts/centimeter. Finally, these studies indicated that electroactivation followed by culture in the presence of cycloheximide for 24 hours was the most efficient treatment for production of pronuclear stage cytoplasm.

Value to the Swine Industry

The production of growth hormone transgenic swine serves as an excellent example of the value of transgenic technology to the swine industry. Production of transgenic pigs from DNA-transformed, individually-derived and screened ES-cell lines would allow large numbers of genetically altered pigs to be established.By utilizing genetically altered ES-cells in nuclear transfer strategies, it may be possible to establish large numbers of genetically identical pigs. Identical animals would be of great value to pork producers for selection of disease resistance, uniformity in production traits and reproductive performance.

Acknowledgements

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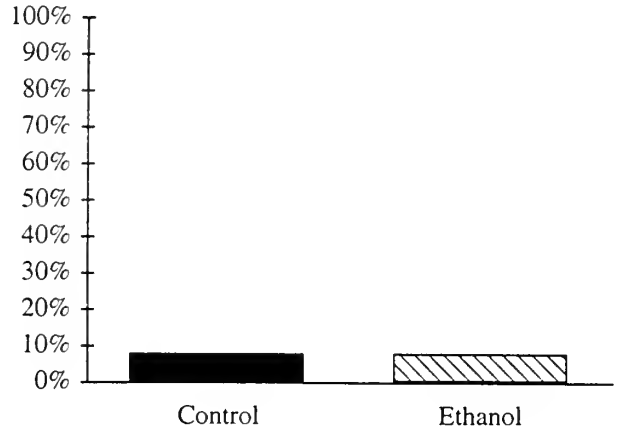

Figure 1. Activation rates for control and ethanol treated porcine oocytes following 20 hours of in vitro culture. Treatments did not differ (P > .05).

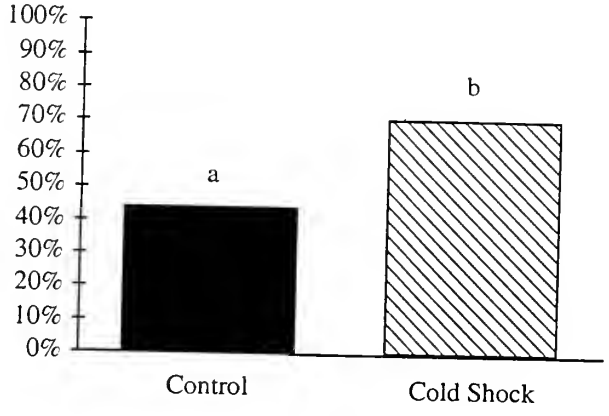

Figure 2. Activation rates for control and cold shock treated porcine oocytes following 20 hours of in vitro culture. [a,b]Bars with different superscripts differ (P < .05).

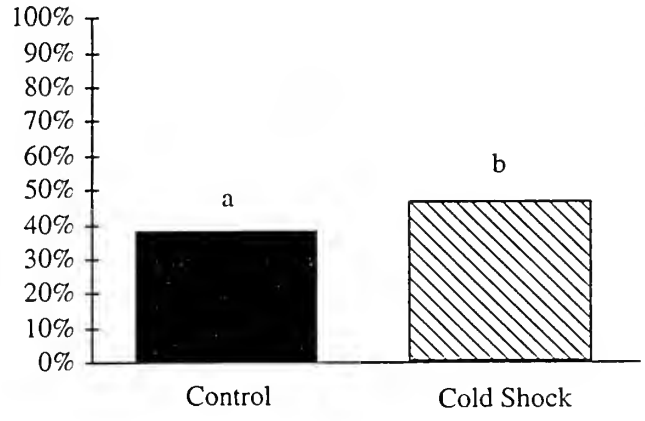

Figure 3. Parthenogenetic two-cell formation for control and cold shock treated porcine oocytes following 20 hours of in vitro culture. [a,b]Bars with different superscripts differ (P < .05).

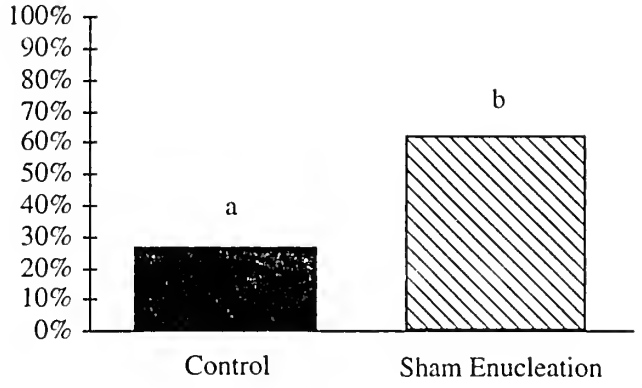

Figure 4. Activation rates for control and sham enucleated porcine oocytes following 20 hours of in vitro culture. [a,b]Bars with different superscripts differ (P < .05).

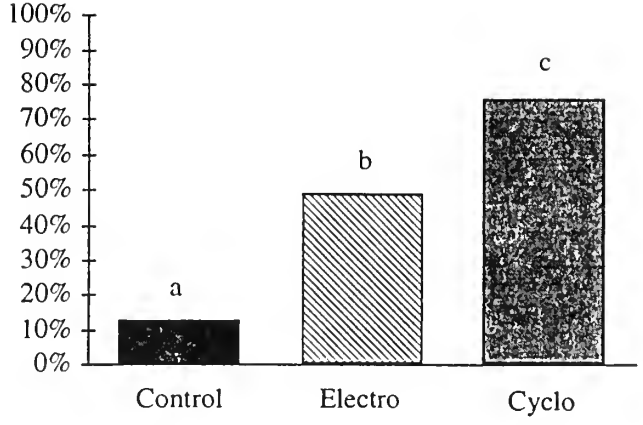

Figure 5. Activation rates for control, electroactivated (Electro) and electroactivated + cycloheximide treated (Cyclo) porcine oocytes following 24 hours of in vitro culture. [a,b,c]Bars with different superscripts differ (P < .01).

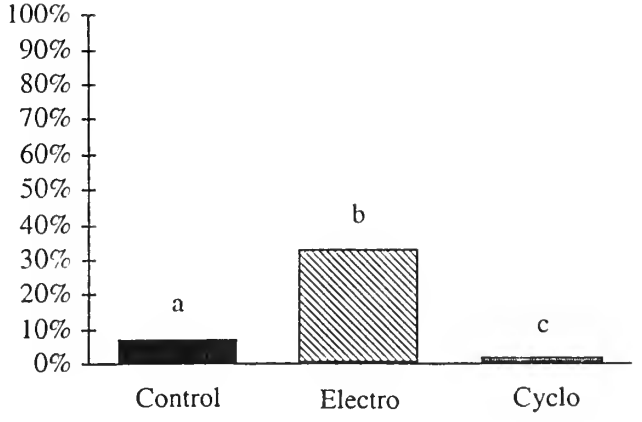

Figure 6. Parthenogenetic two-cell formation for control, electroactivated (Electro) and electroactivated + cycloheximide treated (Cyclo) porcine oocytes following 24 hours of in vitro culture. [a,b,c]Bars with different superscripts differ (P < .05). Parthenogenetic two-cell formation was higher for 2.8 kV/cm than 1.3 kV/cm within the electroactivation treatment (P < .05).

Leptin - Endocrine Signal Regulating the Onset of Post-weaning Estrus in Pigs

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Introduction

Profitability in the swine industry is based on prolificacy. One measurement of prolificacy is the number of piglets produced per year. To optimize prolificacy and profits it is essential that sows are re-bred as soon as possible after weaning. An extended post-weaning anestrus decreases overall productivity and increases costs of production. A large number of sows undergo prolonged post-partum anovulatory periods. Furthermore a significant number of sows, after having their first litter never return to estrus and are removed from the herd. Many factors influence the duration of the weaning-to-estrous interval including: parity, season, duration of lactation, breed and nutritional status (Kirkwood et al.,1984; Aherne and Kirkwood, 1985; Clark et al., 1986; Dial et al., 1987).

Energy Balance

One of the most defined causes of prolonged weaning-to-estrous intervals is negative energy balance (Pettigrew and Tokach, 1993; Jones and Stahley, 1995). Negative energy balance is defined as the use of more energy metabolically than consumption of energy contained in the diet. Animals on a long term energy restricted diet typically have depleted fat stores, very low conception rates and often enter into a period of anovulation (Imakawa et al., 1983; Foster et. al., 1985; Armstrong and Britt, 1987). Similarly during periods of heavy milk production, lactation rather than reproduction takes priority in the partitioning of nutrients. Insufficient feed intake, inadequate energy and heavy milk production cause a large mobilization of energy stores within the animal (Kirkwood et al. 1987a; Kirkwood et al., 1987b). The majority of these molecules are transported to and utilized in the mammary gland for synthesis of milk components. This creates a precarious position for the producer who needs to maintain adequate milk production to sustain the current litter but who also needs to have sows return to estrus promptly after weaning to optimize profitability. Numerous diets and management schemes have been examined in an attempt to reduce the weaning-to-estrous interval. These schemes have improved piglet survivability but prolonged post-partum anestrus still persists.

Nutritional Status and Reproductive Function

Many investigators have examined the relationship between nutritional status, body fat reserves and reproductive function. As a result it has been hypothesized that there is a direct signal or hormone from adipose tissue to the brain or ovary to mediate reproductive function. Adequate nutrition and reserves of the metabolic fuel, fat, are required for ovulation, conception and maintenance of the developing embryo. Animals in a starved or metabolically compromised state are not good reproductive candidates. Logically, there is an evolutionary mechanism which enables the animal to determine if there is enough metabolic fuel or adequate energy storage to

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support the energy demands of pregnancy and lactation. Identification of this signal will enhance our understanding of the mechanisms which control post-partum reproductive function. Better understanding of these mechanisms will enable researchers to develop management strategies, nutritional regimens and/or drug therapies which will improve prolifically and profitability in the swine industry. Therefore our overall goal is to identify and characterize the metabolic signal which regulates reproductive function based on the metabolic state of the individual.

Leptin

Leptin, a newly discovered hormone is the focus of our research. Leptin is hypothesized to be a regulator of satiety, metabolic activity and reproductive function. Adipose tissue synthesizes and secretes leptin into the blood. In feed restricted or malnourished individuals, fat reserves are minimal and concentrations of leptin in the plasma are low. Refeeding or improvement in metabolic status replenishes fat reserves and increases concentrations of leptin in plasma. Similarly, mice that do not produce endogenous leptin are sterile. Injections of leptin reverse this sterility. Several studies have documented the action of leptin on the hypothalamus and its role in regulating reproductive neurohormones and satiety centers (Campfield et al., 1995; Considine et al., 1996; Erickson et al., 1996). However, the ovary appears to also be a target for leptin. Investigators have found that leptin treatment increases the total number of follicles, and synthesis of estrogen (Barash et al., 1996) but decreases synthesis of progesterone in the ovary of rodents in vitro (Spicer and Francisco, 1997). The ovarian leptin receptor has been identified in some species (Cioffi et al., 1997; Zamorano et al., 1997) including pigs (T. Ramsey personal communication, 1997); however its overall function is not known.

At this time we are presenting our research objectives. We do not have current data because the procedures needed to measure leptin in plasma and mRNA for the leptin receptor are being developed.

1) The first specific objective for our research is to determine if concentrations of leptin in plasma of pigs are correlated with body fat composition, stage of lactation and concentrations of protein or energy in the diet. The amount of body fat changes throughout lactation. During peak lactation body fat composition is at its lowest. If adequate dietary energy is not provided during lactation, fat is mobilized from adipose tissue and breakdown of body protein in skeletal muscle occurs (Pettigrew and Tokach, 1993; Jones and Stahley, 1995). These products are then used to provide the necessary energy needed to maintain lactation. Typically fat and protein stores are broken down in a 1:1 ratio (Burlacu, 1983). However, diets containing adequate protein but low energy cause depletion of fat stores but no breakdown of body proteins (Garlic et al., 1980). Since adipose tissue synthesizes leptin and secretes it into the circulation, any physiological process affecting the amount of adipose tissue could affect concentrations of leptin in plasma.

Blood was collected from 72 primiparious sows on days 1, 7,14,21 and 28 post partum with 8 sows per day. These sows were fed one of four diets containing either: 1) Control (HE-HP), 2) high protein, low energy (HP-LE); 3) low protein, high energy (LP-HE); or 4) low protein, low energy (LP-LE) diets during lactation. Concentrations of leptin in these samples will be determined and compared statistically with whole body fat compositions, diet and stage of

lactation. We expect that body composition will be related positively to diet and that concentrations of leptin in plasma will be positively correlated with body fat. As fat within the carcass increases, concentrations of leptin in the plasma increases. We also expect that as lactation progresses, body fat composition will decrease which results in decreased concentrations of leptin in the plasma. Animals being fed HE-HP (control) diets should be adequately fed and should be mobilizing very little body stores, particularly during late lactation. These animals then should have relatively high concentrations of leptin in plasma. Animals being fed HE-LP diets should be mobilizing protein stores rather than fat stores. Therefore, these animals should have similar body fat composition and concentrations of leptin in plasma as the animals on the HE-HP(control) diet. Animals being fed LE-HP diets will be mobilizing large amounts of body lipids (fats) but very little protein stores. Leptin concentrations should be lower in these animals because of the large decrease in body fat composition. Similarly, animals being fed LE-LP will be mobilizing large energy reserves including both protein and fat. As fat stores are utilized, concentrations of leptin should decrease.

2) Our second objective is to determine if loss of body fat stores during lactation affects expression of the leptin receptor in the ovary. Approximately ten years ago we developed a nutritional model which causes significant fat loss during lactation and extended post-weaning anestrus. These physiological changes are similar to those observed in many pigs during lactation, especially those having their first litters. Loss of fat stores equates to a loss of adiposity which should result in decreased concentrations of leptin in plasma. As mentioned before, sows with minimal body fat stores are reproductive quiescent. Leptin may exerts its effects on either the brain, ovary or both. If different concentrations of leptin in plasma affect the ovary directly, it is anticipated that leptin receptor expression in the ovary would change with varying concentrations of leptin in plasma.

Twenty primiparious pigs will be randomly assigned to either a control or low energy diet at parturition (n=10/group). Litters will be standardized to 8 per sow. Blood will be collected on day 0 (parturition), day 7, day 14, day 21 and day 23 post-partum and analyzed for concentrations of leptin. Litters will be weaned on day 21. Sows will be sacrificed on day 23 and ovaries will collected and frozen rapidly. Total RNA will be isolated within a day of collection. Expression of the leptin receptor will be analyzed using Northern Blot analysis. Blots will be standardized using 28S ribosomal RNA, scanned and density of hybridization signal expressed as a % of control. Results between treatments will be statistically examined using a one-way analysis of variance with leptin as a co-variate (SAS, 1985).

Our studies are a step toward a better understanding of the controls of post-partum anestrus in sows. Leptin may be the metabolic signal that shuts off reproductive function in response to poor nutritional status. Further investigation needs to be conducted involving the direct effects of leptin in the ovary and its role as a whole in reproduction.

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Transgenic Alteration of Sow Milk to Improve Piglet Growth and Health

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Inadequacies of Sow Lactation

In intensive pig production systems, piglets achieve only about half their potential growth-rate during a lactation period.

Rationale

The amount and composition of milk that a sow produces has a significant impact on the growth, health and development of her offspring. Piglet growth and health influences all subsequent aspects of pork production. In swine, 44% of the growth of suckling pigs is accounted for by the volume and solids content of the mother's milk. Low milk production decreases piglet growth rate and results in decreased piglet weaning weight whether the piglets are weaned at 21, 14 or 7 days of age. This decreased weaning weight affects overall pig performance through the nursery, grower and finishing stages and also affects general piglet health. Decreases in weaning weight affect subsequent reproductive performance of females resulting in prolonged weaning to first estrus interval and lowers the ovulation rate and subsequent litter size.

Lactational efficiency may ultimately affect sow longevity in the herd, because a number of sows are culled from herds due to poor lactational performance. Current swine management schemes attempt to maximize the number of piglets born per litter and piglet survival. In order to utilize the larger litter sizes and keep the piglets alive and healthy, maximum milk production must be obtained. Indeed, the gains that have been made in decreasing newborn mortality combined with the increased litter sizes from selected high genetic merit sows make milk production and milk composition one of the most important limiting factors in piglet survivability and growth.

Project Objectives

1. To study the effects of increasing α-lactalbumin levels on milk production, milk composition, piglet health and survivability in sows transgenic for the bovine α-lactalbumin gene.

2. To generate transgenic swine that produce higher levels of the growth factor IGF-I in their milk and examine its effects on the growth, health and development of the piglets.

Introduction

Transgenic animals are animals that contain a gene or engineered gene that they would not have obtained through normal breeding or mating practices. The genes are transferred into the animals by injecting the gene of interest (DNA) into a developing embryo. In a small percentage of the embryos, the injected gene inserts into the DNA of the animal and becomes part of the animal's genome. This allows the gene to behave like any other of the animals genes and the gene is passed on to the offspring in a normal fashion.

α-Lactalbumin is a major whey protein of cow and pig milk. Within the mammary gland, α-lactalbumin combines with β1,4 galactosytransferase to form the enzyme lactose synthase. Lactose synthase produces the main milk carbohydrate, lactose. Lactose also is the major osmole in milk causing water to be transported into milk.

Our hypothesis is that if more lactose is produced, the lactose will draw water by osmosis into milk and increase milk volume. Thus, we propose that increasing the level of α-lactalbumin in pigs will result in greater lactose production and greater milk volume. We have previously found that this occurs in mice.

Insulin-like growth factor I (IGF-I) is a bioactive protein found in the milk of mammals and at significantly higher levels in colostrum than in later stage milk. When IGF-I is given to piglets in milk replacer, it results in greater intestinal growth and maturation. IGF-I also increases the levels of intestinal enzymes. Based on these results, we wish to test the potential beneficial effect that higher levels of IGF-I in the milk may have on piglet growth, intestinal health and the weaning transition.

Results

Two lines of transgenic pigs containing the bovine α-lactalbumin gene have been produced. One of the lines is currently being studied, while the second line is being propagated to allow further investigation. The first line of pigs produce bovine α-lactalbumin in their milk at a concentration of 0.07%. This is approximately one-half the normal level of porcine α-lactalbumin found in milk, so we have increased the total α-lactalbumin production in these sows by 50% from approximately 0.14% to 0.21%. The effects that this increase has on milk production in the sows and on the growth of their piglets is being examined.

A transgenic boar containing a gene construct that will allow production of higher IGF-I levels in milk has been produced. He is currently being mated to produce transgenic sows that will allow us to study the effects of this gene on piglet growth and health. In addition to the initial boar, three additional transgenic boars have recently been produced with this same IGF-I gene construct.

Potential Impacts on the Swine Industry

1. Increasing sow milk production 10% would result in an additional $2.46 per litter weaned (Average litter size 7.8 piglets).

2. The increased weaning weight will improve the weaning transition and shorten the number of days to market or puberty.

3. Increasing the amount of IGF-I in sow's milk will improve the intestinal health of the piglets.

4. Improved intestinal health may improve the weaning transition and also decrease the susceptibility to intestinal diseases.

Comparison of Feed Intake Patterns of Group-Housed Yorkshire and Meishan x Yorkshire Pigs

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Introduction

There is considerable variation among conventional breeds and lines of pigs for feed intake and growth rate (Cöp and Buiting, 1977; Miller et al., 1997). Cöp and Buiting (1977) reported differences in daily feed intake between six European lines of pigs increased from 150 g at 25 kg to more than 500 g at 95 kg live weight. In addition, there is evidence of breed variation in feed intake patterns. For example, De Haer and De Vries (1993) showed that Dutch Yorkshire pigs ate more frequently and faster than Dutch Landrace pigs, and concluded that differences in feed intake pattern contributed, in part, to observed differences in lean percentage between these to breeds.

A breed that has received considerable recent interest in Europe and the United States of America is the Meishan, which differs markedly from conventional genotypes for all production traits, including feed intake and growth rate. In addition, there is evidence that the Meishan has different behavior patterns than other breeds of pigs. For example, French research showed that when Meishans were exposed to a new environment they vocalized less, had lower locomotor activity, and lower food intake latency after 24 hours of food deprivation than other Chinese breeds (Mormede et al., 1984). Hyun et al. (1996), using electronic feed intake recording equipment, showed substantial differences between purebred Yorkshire and Meishan barrows for feed intake and feeding behavior when they were reared in mixed-genotype groups, with Yorkshires making more frequent visits to the feeder and having higher feed consumption rates. However, one possible explanation for the differences in feeding pattern between these breeds in the study of Hyun et al. (1997) is that the Yorkshire pigs were more dominant and, consequently, this restricted the feeding behavior of the Meishans. The objective of the current study was to compare the feed intake, growth performance and feeding patterns of Yorkshire and Meishan x Yorkshire growing pigs, penned in either mixed- or single-genotype groups, using electronic feed intake recording equipment.

Experimental Approach

The pigs used in this study were produced from sows that had been inseminated with a mixture of Meishan and Yorkshire semen and, therefore, both genotypes were born in the same litter and had common dams. Yorkshire and Meishan x Yorkshire pigs were reared in either single- or mixed-genotype groups. Single- genotype groups were defined as the pens containing either only Yorkshire or only Meishan x Yorkshire pigs. Mixed-genotype groups were defined as the pens containing an equal number of both Yorkshire and Meishan x Yorkshire pigs. There were two pens of pigs per treatment and equal numbers of barrows and gilts across genotypes and pens. A single diet was fed during the study period. This was formulated using corn and soybean meal using the nutrient requirement recommended by NRC (1988) and contained 17% crude protein and 3,365 kcal/kg ME. The study was carried out in grower accommodation that

had partially slotted concrete floors and the space allowance was 8ft^2/pig. The accommodation had continuous lighting and water was available via a nipple drinker in each pen. The temperature within the building was maintained at 22° C using a thermostat and fan ventilation. The trial started at an average pen live weight of 28.5 kg and ended after a 5-week period at which stage the average pen weight was 52.0 kg.

The pigs were given *ad libitum* access to feed using Feed Intake Recording Equipment (F.I.R.E., Osborn Industries, Osborn, Kansas) which recorded feed intake and feeding pattterns. Each of the pens was equipped with a feed station which consisted of a feed trough connected to a load cell and receiving equipment that identified signals from the ear tag transponder carried by the animals. Pigs had 24-h access to the feed station which was equipped with a full-length protective crate in front of the feed trough to prevent access to the trough by more than one pig at any time. All feed stations were connected to control equipment which continuously logged the time and duration of each feeder visit, and the weight of feed consumed per visit as well as cumulative feed intake for an individual pig over a 24-h period. Data were downloaded from the control equipment memory on a daily basis and stored on diskette until required for analysis.

Results and Discussion

The feed intake and growth performance of the pigs is summarized in Table 1. There were no statistically significant differences in growth performance between the two genotypes used in this study. A number of studies have compared the Meishan with conventional breeds, such as the Yorkshire, and have generally shown that growth rates for Meishans are approximately 50 to 65 % of those for the conventional breed (Haley et al., 1992). However, the F1 crosses of Meishan and conventional breeds have growth rates that are more similar to those for conventional breeds.

Daily means for feeding pattern are summarized in Table 2. There were no statistically significant interactions between genotype and group composition for any of the traits. However, there were a number of differences between the genotypes for feeding pattern which were independent of group composition. Meishan x Yorkshire pigs made fewer feeder visits but spent more time in the feeder and consumed more feed at each visit than Yorkshire animals but, overall, total feeder occupation times, feed consumption rates and feed intakes were not different between the genotypes. Hyun et al. (1997) compared the feeding patterns of purebred Meishan and Yorkshire animals and showed similar differences in respect of the number and duration of visits and feed consumed per visit. However, these authors also showed that Meishans had slower feed consumption rates and greater total daily feeder occupation times compared to Yorkshires. In the present study, there was a trend for Meishan x Yorkshire animals to have slower feed consumption rates than Yorkshires (13.6 vs. 15.1 g/min, respectively; P=0.18), however there was no difference among the genotypes for total daily feeder occupation time. The magnitude of the genotype differences were much greater in the study of Hyun et al. (1997) than in the present experiment which is to be expected given that the two studies used purebred Meishans and Meishan x Yorkshires, respectively. The feed intake patterns of barrows and gilts were similar which is also in agreement with the study of Hyun et al. (1997).

Pigs showed a diurnal feeding pattern with the general pattern of feeder visits over the 24-h period being similar for both genotypes (Figure 1). Feeder visits were lowest during the nighttime between approximately 1800 and 0600 h and highest during the daytime, particularly between 0800 and 1400 h (Figure 1). Meishan x Yorkshire pigs made fewer feeder visits than

Yorkshire animals throughout the 24-h period, with the difference between the genotypes being statistically significant for every hour, with the exception of three hours in the evening and one hour in the early morning (Figure 1).

The lack of any interaction between genotype and group composition in the current study indicates that the genotype differences in feeding pattern were similar in single- and mixed-genotype groups. However, animals in mixed-genotype groups made fewer but longer feeder visits and had longer daily feeder occupation times which suggests that some factor was altering feeding behavior when the two genotypes were penned together compared to when they were in single-genotype groups. Never-the-less, feed intakes and growth rates were similar for single- and mixed-genotype groups and the differences in feeding pattern were relatively modest. Our original hypothesis was that in mixed-genotype groups Yorkshire pigs will be dominant to Meishan x Yorkshire and that this will result in changes in feeding pattern in Meishan x Yorkshire. This hypothesis is not substantiated by the results of the present study which suggest that there is a genetic difference between the Meishan x Yorkshire and Yorkshire which is independent of the genetic composition of the group. These two breeds are examples of the extremes in terms of biological and production characteristics and exhibit differences in most traits. However, there is evidence of differences in feeding patterns among conventional breeds and lines (De Haer and Merks, 1992; De Haer and De Vries, 1993). Further study of the genetic basis of feeding pattern and its relationship with growth performance would appear warranted.

Conclusions

1. Yorkshire pigs made more frequent visits to the feeder and ate less per visit when compared to the Meishan x Yorkshire. However, the differences between these two genotypes were less than those found in a previous studies which compared purebred Yorkshire and Meishan pigs.

2. This study suggests, therefore, that there are substantial differences between genotypes in feeding pattern. However, the relevance of these differences for practical swine improvement programs and production systems is unclear and warrants further research.

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Table 1. Least squares means for genotype, group composition, and sex for feed intake and growth performance.

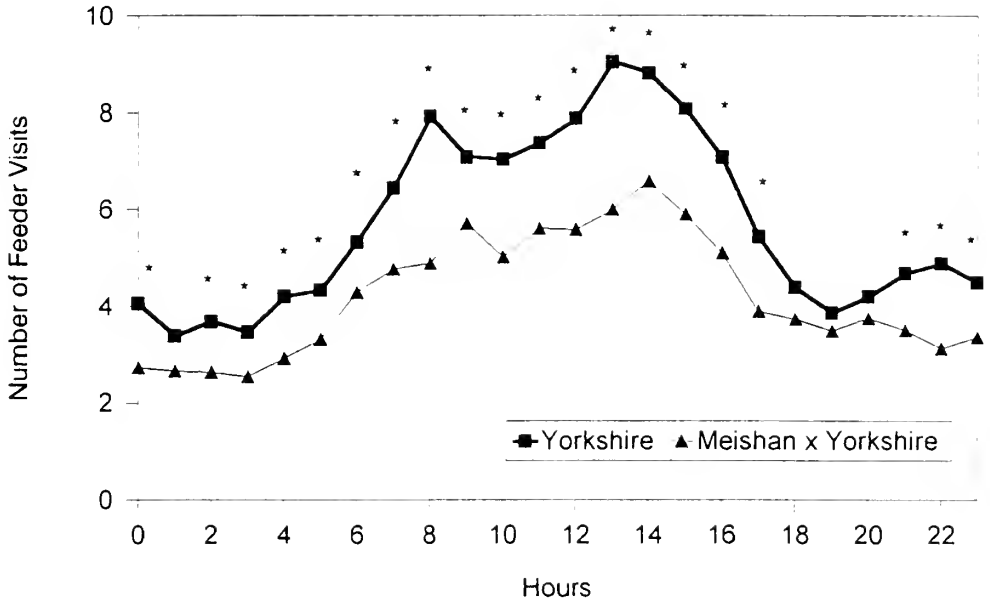
	Treatment						
	Genotype		Group Composition		Sex		Average
Item	Yorkshire	Meishan x Yorkshire	Single Genotype	Mixed Genotype	Barrows	Gilts	SEM
Initial body weight (kg)	28.5	28.6	28.6	28.5	28.1	29.0	0.71
Final body weight (kg)	53.0	51.1	52.3	51.8	51.7	52.5	1.10
Daily feed intake (kg)	1.58	1.44	1.51	1.52	1.60	1.43	0.068
Average daily gain (g)	700	644	676	667	674	670	19.9
Gain : Feed	.44	.44	.45	.43	.43	.45	0.094

Table 2. Least squares means for genotype, group composition, and sex for feeding behavior.

Item	Treatment						Average
	Genotype		Group Composition		Sex		
	Yorkshire	Meishan x Yorkshire	Single Genotype	Mixed Genotype	Barrows	Gilts	SEM
Daily feed intake (kg)	1.58	1.44	1.51	1.52	1.60	1.43	0.07
Number of feeder visits per day	17.1[a]	12.3[b]	15.8[a]	13.6[b]	15.7	13.7	0.85
Feed intake per visit (g)	101[a]	124[b]	107	117	111	114	5.8
Feeder occupation time per visit (min)	7.11[a]	8.80[b]	7.22[a]	8.69[b]	7.64	8.27	0.342
Feeder occupation time per day (min)	109	105	100[a]	113[b]	110	104	4.6
Feed consumption rate (g/min)	15.1	13.6	15.2	13.5	14.9	13.8	0.75

[a,b] Means within the same row within treatment with different superscripts are significantly different ($P < 0.05$)

Figure 1. Comparison of Yorkshire and Meishan x Yorkshire for number of feeder visits by hour of day.



* Denotes means within each hour that differ ($P<0.05$)

Influence of Sire Line and Halothane Genotype on Growth Performance, Carcass, and Meat Quality Characteristics in Pigs.

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Introduction

The choice of sire line is critical in dictating the performance levels achieved on a swine enterprise. However, there is a dearth of information in the scientific literature on the relative performance of commercially available sire lines. In addition, most sire line comparisons have focused largely on growth and carcass traits and there is limited data on meat quality and processing characteristics. Currently, producers have the option of using sire lines of different halothane status, namely reactor, carrier or negative animals. The halothane gene is of interest because of its positive effects on carcass traits (Aalhus et al., 1991; Pommier et al., 1992) and negative effects on meat quality (Sather et al., 1991; Jones et al., 1994). The development of the HAL-1843 DNA test (Fujii et al., 1991; Otsu et al., 1991) which distinguishes between animals that are homozygous or heterozygous for the halothane mutation has allowed within-litter comparison of the various halothane genotypes to be carried out (Leach et al., 1996), which results in the effects of this gene being measured against the same genetic background.
The current study was carried out to evaluate the growth, carcass, and meat quality characteristics of three commercial sire lines from one genetic source that represented the range in growth rates likely to be available to commercial producers from this supplier. In addition, a within-litter evaluation of the effects of the halothane gene was also undertaken.

Materials and Methods

Trial Design and Performance Test. The study was conducted at the Swine Research Center at the University of Illinois. Progeny from three commercial sire lines, supplied by the Pig Improvement Company, U.S.A. (Franklin, KY) and mated to the same halothane negative hybrid dam line (Camborough 15), were compared. Sire line A was a halothane reactor line and produced halothane carrier progeny; line C was halothane negative and produced negative progeny; line B was a cross between lines A and C and was a halothane carrier that produced both carrier (BNn) and negative (BNN) progeny within the same litter. Within each sire line, 6 boars were used and these were mated to an average of three sows per boar, using artificial insemination. Blood samples were obtained from the progeny of the line C sires at approximately 4 weeks of age and the halothane status of each animal was identified using the HAL-1843 DNA test.

A total of 160 pigs, with equal numbers of barrows and gilts, were put on test at a live weight of approximately 40 kg over a period of six weeks. Pigs were allocated on the basis of weight to like-sire line, like-halothane genotype, like-sex groups of four pigs. During the study, pigs were housed in a controlled environment finishing house which had partially slatted floors and were provided a pen space allowance of 1.2 m^2 per pig. Animals were given ad libitum access to diets based on corn and soybean meal from a two-hole feeder. A two-phase dietary program was used with the first phase diet being fed between 40 to 80 kg and formulated to supply 18.2 % protein and 3384 kcal/kg ME. The second phase diet was fed between 80 to 120

kg and was formulated to supply 16.5 % protein and 3390 kcal/kg ME. Pigs and feeder were weighed weekly and feed additions to the hoppers were recorded.

Pigs were taken off test when they were within 3 kg of the designated slaughter weight of 115 kg at the weekly weighing. Pigs remained in their test groups until they were despatched from the farm on the afternoon prior to slaughter and transported to the Meat Science Laboratory at the University of Illinois where they were held for approximately 16 h before slaughter. Animals were mixed with those from other groups during transport and in the lairage where they were held overnight without food but with access to water.

Slaughter and Carcass Evaluation. One hundred and fifty-four pigs reached the designated slaughter weight and were slaughtered for carcass and meat quality evaluation. At 24 hours postmortem, carcass measurements were obtained on the left side of each carcass. The right side of the carcass was weighed, fabricated using the procedures described by NAMP (1992), and weights recorded for carcass cutting yields. Trimmed, boneless, wholesale cuts were obtained and the weights were recorded. The trimmed, boneless product from the ham, shoulder, loin, belly, and the soft tissue were ground separately through a 3 mm plate in a meat grinder and further homogenized in a food processor. The samples were frozen and held at -20°C for subsequent chemical analysis for fat-free lean determination.

Meat Quality Measurements. Longissimus pH was measured at 45 min and 24 h postmortem. Longissimus color, firmness, marbling, were also taken using the procedures described by NPPC (1991). Loin chops (2.5 cm thick) were obtained from the longissimus immediately posterior to the last rib from the left side of the carcass. One chop was weighed, placed in a Whirl-pak bag, and suspended in a 40°C cooler for 24 h then reweighed and drip loss recorded. The remaining three chops were vacuum packaged, aged 7 d at 20°C, and frozen (-20°C) for subsequent use in sensory evaluation and Warner-Bratzler shear force determination.

Shear Force and Sensory Evaluation. Chops for Warner-Bratzler shear force were thawed for 24 h at 40°C and then cooked on Farberware open hearth grills to an internal temperature of 70°C. Temperature was monitored using copper Constantan thermocouples and a recording thermometer. Chops were weighed before and after cooking to determine cooking loss. Chops were cooled to 25°C and six 1.3 cm diameter cores were removed parallel to the muscle fibers and used for Warner Bratzler shear force measurements. Chops for sensory evaluation were prepared and cooked using the same procedures as for shear force. A taste panel consisting of 6 trained panelists evaluated juiciness, tenderness, and off-flavor intensity using a 15 cm structured line scale with anchors and a mid-point (0 cm = extremely dry, tough, and intense off-flavor to 15 cm = extremely moist, tender and no off-flavor).

Results and Discussion

There were a small number of interactions between sire line and sex which were of limited practical importance and have not been reported. In addition, the differences between barrows and gilts were similar to those found in other studies and, therefore, only the sire-line means are presented.

Growth Performance. The means for growth performance are presented in Table 1. Line A pigs consumed less feed and grew slower ($P < .01$) compared to the other lines which were similar in these respects. However, there was no differences among the lines in gain:feed ratio.

Slaughter and Carcass Measurements. The sire line means for slaughter and carcass characteristics are presented in Table 2. Fasted live weight, taken immediately prior to slaughter, was different between the lines and, therefore, all subsequent carcass measurements have been corrected for slaughter weight by covariance. Hot carcass weights and dressing percentage were lower for line C than for the other lines which were similar in these respects. The line differences in dressing percentage, which were between 1.69 and 2.31 percentage units (Table 2), are of considerable economic importance to swine producers that are paid for pigs on a dead weight basis. Within line B, the difference between the halothane genotypes for dressing percentage was small and not statistically significant. Linear carcass measurements did not differ among sire lines and within line B, among the halothane genotypes, (Table 2). Sire line means for fat-free lean content of the side are presented in Tables 3, respectively. There was no difference between the sire lines for fat-free lean weight or percentage (Table 3). The current study, therefore, suggests little effect of the halothane gene on carcass lean content, which further demonstrates the need to remove the gene from present swine populations.

Meat Quality Measurements. Sire line means for meat quality measurements taken on the longissimus are presented in Table 4. The muscle pH measured at 45 min postmortem was lower for line B carriers than for the two halothane negative populations (line B and C). However, there was no difference between line B carrier and negative animals for subjective color, firmness, and marbling, or drip loss (Table 4). The color and firmness of the muscle postmortem is a function not only of halothane genotype but also pre-slaughter handling procedures and postmortem carcass chilling rates and, thus, variation between studies in the relative differences between carrier and negative animals for muscle quality characteristics may reflect differences in pre- and postmortem conditions.

There were significant differences between the lines for shear force and taste panel tenderness and juiciness (Table 4) with line A having higher shear force and lower tenderness and juiciness values than line C indicating poorer eating quality for line A progeny. Within line B, carriers had lower juiciness scores than negatives. In addition, tenderness scores were lower and shear force values were higher for line B carriers compared to line B negative pigs, although these differences were not statistically significant (Table 4). Thus, the two halothane carrier populations used in this study produced poorer eating quality than the two negative populations. There is evidence that PSE meat has poorer palatability traits than normal pork (Topel et al, 1976) and halothane carriers can have a higher incidence of PSE than negative animals, therefore, poorer eating quality might be anticipated for carriers compared to negative animals. However, in this particular study, muscle color and drip loss were not markedly different between carrier and negative pigs suggesting a similar incidence of the PSE condition in the various populations. On this basis, it would seem unlikely that the variation between the lines in eating quality observed in this study can be attributed to the halothane gene per se. Interestingly, more recent studies with the progeny of line C sires has shown the presence of a sub-population of animals with enhanced eating quality, which also have high values for muscle glycolytic potential (Sutton, 1997). European studies have shown the presence of a single gene, the so-

called Rendement Napole, or RN gene (Monin and Sellier, 1985; Naveau, 1986; Fernandez, 1992), in pure- or crossbred Hampshire populations which is also associated with high muscle glycolytic potential and enhanced eating quality (Lundstrom, 1996). It is possible, therefore, that the improved eating quality observed for lines C and BNN in the present study results from the presence of the RN gene in these populations rather than the halothane gene. The RN gene produces relatively low muscle ultimate pH values and in the current study 24-h pH values were lower for line C animals, although this difference was not statistically significant ($P<.10$). However, glycolytic potential values were not available for the pigs used in the present study and further research comparing the possible combinations of genotypes at the RN and halothane loci is required to determine the relative effects of these two genes on eating quality.

Conclusions

1. Line A progeny consumed 400 g / day less feed, grew 200 g /day slower, and took approximately 14 days longer to reach market weight. However, there were no differences among the sire lines or between halothane carrier and negative animals in feed efficiency.

2. Substantial differences in dressing percentage were observed among the sire lines of up to 2.3 percentage units. Such differences in carcass are of substantial great economic importance to producer who sell pigs on the basis of dead weight.

3. No differences in carcass leanness were detected across sire lines or halothane genotypes.

4. The impact of the halothane gene on carcass and meat quality traits was relatively limited and much less than observed in most previous studies.

5. There were considerable differences among the sire lines for eating quality traits such as tenderness, and juiciness are most likely the result of genes other than the halothane gene.

6. The substantial differences in growth rate, carcass characteristics and meat quality among the sire lines used in this study highlight the importance of considering all of these aspects when choosing genetic lines for use in particular production systems and marketing schemes.

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Table 1. Least squares means for growth performance

Sire line	A	B	B	C		
Halothane Genotype	Carrier (Nn)	Carrier (Nn)	Negative (NN)	Negative (NN)	Ave SE	Sig [c]
Weight at start of test, kg	40.79	39.70	38.05	40.61	.79	NS
Weight at end of test, kg	111.40	114.32	113.47	113.25	1.35	NS
Days on Test	80.25[a]	70.80[b]	72.20[b]	67.42[b]	1.87	**
Average daily gain, kg	.885[a]	1.057[b]	1.049[b]	1.082[b]	30	**
Gain:feed	.38	.39	.37	.39	.01	NS
Average daily feed intake, kg	2.37[a]	2.72[b]	2.82[b]	2.79[b]	.08	**

1 kg = 2.2046 lbs

[a, b] Means within a row with differing superscripts differ, P < .05.

[c] ns, **, = not significant, P < .01, respectively.

Table 2. Least squares means for slaughter and carcass measurements

Sire line	A	B	B	C		
Halothane Genotype	Carrier (Nn)	Carrier (Nn)	Negative (NN)	Negative (NN)	Ave SE	Sig [c]
Fasted live weight, kg	114.65[ab]	115.67[a]	113.02[b]	115.55[a]	.62	0.00
Hot carcass wt, kg	88.74[a]	89.32[a]	88.56[a]	86.63[b]	.42	***
Dressing percent (hot)	77.42[a]	77.62[a]	77.00[a]	75.31[b]	.38	***
Carcass length, cm	83.23	83.24	82.34	84.22	.53	NS
Midline fat measurements, cm						
First rib	3.92	3.90	4.05	3.77	.15	NS
Last rib	2.55	2.41	2.70	2.28	.10	NS
Last lumbar	1.97	2.01	2.38	1.79	.14	NS
Tenth rib measurements, cm						
Fat depth ½	2.32	2.36	2.40	2.15	.10	NS
Loin eye depth ½	6.22	6.20	6.28	5.90	.14	NS
Loin eye area, cm^2	42.17	43.25	42.44	38.42	1.14	NS

1 kg = 2.2046 lbs; 1 cm = .3937 in

Measurements corrected for live weight, kg.

[a, b] Means within a row with differing superscripts differ, $P < .05$.

[c] ns, *, **, ***, = not significant, $P < .05$, $P < .01$, $P < .001$, respectively.

[d] Subjective score from 1 = thin, to 3 = thick.

Table 3. Least squares means for side fat-free lean weights and percentages

Sire line	A	B	B	C		
Halothane Genotype	Carrier (Nn)	Carrier (Nn)	Negative (NN)	Negative (NN)	Ave SE	Sig [c]
Fat-free Lean Weights (kg)						
Shoulder	5.83	5.67	5.51	5.63	.13	NS
Ham	6.86[a]	6.65[ab]	6.36[ab]	6.27[b]	.17	*
Loin	4.52	4.56	4.25	4.30	.13	NS
Belly	3.46[ab]	3.76[a]	3.33[a]	3.67[ab]	.11	*
Soft Tissue	3.54	3.51	3.54	3.34	.18	NS
Fat-free Lean Percentage [d]						
Shoulder	13.55	13.11	12.86	13.41	.29	NS
Ham	15.94	15.39	14.79	14.93	.37	NS
Loin	10.48	10.55	9.95	10.26	.29	NS
Belly	8.03[ab]	8.70[a]	7.80[a]	8.77[b]	.25	*
Soft Tissue	8.24	8.13	8.30	7.96	.41	NS
Side Fat-free Lean						
Weight, kg	23.84	24.27	23.19	23.33	.40	NS

Percentage [d]	55.53	56.16	54.52	55.59	.79	NS

1 kg = 2.2046 lbs

Measurements are corrected for live weight, kg.

[a, b] Means within a row with differing superscripts differ, P < .05.

[c] ns, * = not significant, P < .05, respectively.

[d] Percentage of side weight.

Table 4. Least squares means for meat and eating quality measurements taken on the longissimus

Sire line	A	B	B	C		
Halothane Genotype	Carrier (Nn)	Carrier (Nn)	Negative (NN)	Negative (NN)	Ave SE	Sig [c]
pH 45 min.	6.15[ab]	6.03[b]	6.28[a]	6.23[a]	.06	*
pH 24 hrs.	5.68	5.68	5.70	5.47	.07	NS
Color [d]	2.28	2.44	2.54	2.36	.21	NS
Firmness [d]	2.22	2.17	2.58	2.37	.12	NS
Marbling [d]	1.30	1.41	1.76	1.38	.16	NS
Drip Loss, %	4.73	5.07	4.89	5.34	.68	NS
W-B shear force, kg	4.32[a]	3.57[b]	3.21[b]	3.30[b]	.17	**
Cooking Loss, %	26.10	26.64	23.30	25.11	4.90	NS
Juiciness [e]	7.60[a]	7.15[a]	8.75[b]	8.76[b]	.43	**
Tenderness [e]	7.54[a]	7.77[ab]	9.09[b]	9.81[b]	.79	*
Off-flavor [e]	14.30	14.37	14.49	14.10	.15	NS

1 kg = 2.2046 lbs

[a, b] Means within a row with differing superscripts differ, P < .05.

[c] ns, *, ** = not significant, P < .05, P < .01, respectively.

[d] Subjective score from 1 = extremely pale, soft, and devoid of marbling to 5 = extremely dark, firm and abundant marbling.

[e] Subjective score from 0 = extremely dry, tough, and intense off-flavor to 15 = extremely moist, tender and no off-flavor.

Relationships between Glycolytic Potential and Growth Performance, Carcass and Meat Quality Characteristics within a Swine Population with Low Glycolytic Potential.

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Introduction

The swine industry has focused on providing consumers with a consistent, high quality product. Because of this much research has focused on factors which affect meat quality. One genetic factor, which is of considerable current interest in the Rendement Napole (RN) or acid meat gene. The effects of the RN gene were first observed in the Hampshire breed by Sayre et al. (1963) and Monin and Sellier (1985) who both reported Hampshire sired pigs as having lower ultimate pH values. LeRoy et al. (1990) and Fernandez et al. (1992) later reported that the effects exhibited by pigs of Hampshire descent were the result of a single dominant gene, which currently appears to be Hampshire breed specific. This gene was called the Rendement Napole or RN gene with the negative and positive alleles being designated RN^- and rn^+, respectively, The effects of the negative allele are due to elevated glycogen levels within the muscle, which in turn are converted into lactate postmortem. The elevated lactate levels produce acidic meat, which has decreased water holding capacity, and increases purge loss. Monin and Sellier (1985) developed reported a method to distinguish between the RN genotypes based on muscle glycolytic potential levels. Glycolytic potential is representative of the metabolites involved within the glycolysis pathway which have the potential to be transformed into lactate. To determine glycolytic potential, a muscle sample from the longissimus must be recovered by live animal biopsy or from the carcass postmortem. Classification of animals as RN^- homozygotes and carriers or rn^+ homozygotes can be determined by plotting the frequency distribution of glycolytic potential for a population of pigs (Fernandez et al., 1992). If the gene is segregating in the population then a bimodal distribution is observed with individuals in the lower distribution being homozygous positive (rn+rn+) and having normal glycolttic potential. Animals in the upper distribution are either homozygous negative (RN^-RN^-) or carriers (RN^-rn^+), have elevated glycilytic potential and have reduced water holding capacity. A number of studies have compared Hampshire populations with normal or elevated glycolytic potential. However, the relationship between glycolytic potential and its components and economically important traits within a population with normal glycolytic potential has not been established.

Materials and Methods

Trial design and performance test. The study was conducted at the Swine Research Center at the University of Illinois. Progeny from one commercial sire line of Hampshire ancestry, supplied by the Pig Improvement Company (Franklin, KY), and mated to a hybrid dam line (Camborough 22), were evaluated.

A total of 96 pigs, with equal numbers of barrows and gilts were put on test at a live weight of approximately 40 kg. Pigs were allocated on the basis of weight to mixed-sex groups of twelve pigs. During the study, pigs were housed in a controlled environment finishing house which had partially slatted floors and were given a pen space allowance of 1.2 m^2 per pig. Animals had ad libitum access to corn - soybean meal based diets from a two-hole feeder. A

two-phase dietary program was used with the first phase diet being fed between 40 to 60 kg and formulated to supply 17.3 % protein and 3299 kcal/kg ME. The second phase diet was fed between 60 to 120 kg and was formulated to supply 15.8 % protein and 3329 kcal/kg ME.

Pigs were taken off test when they were within 3 kg of the designated slaughter weight of 120 kg. When only one pig remained in a pen it was taken off test and slaughtered. Pigs remained in their test groups until they were despatched from the farm on the afternoon prior to slaughter and transported to the Meat Science Laboratory at the University of Illinois where they were held for approximately 16 hours before slaughter. Animals were mixed with those from other groups during transport and in the lairage where they were held overnight without food but with access to water.

Slaughter and carcass evaluation. Ninety-four pigs reached their designated slaughter weight and were slaughtered for carcass and meat quality evaluation. At 24 hours postmortem, carcass measurements were obtained on the left side of each carcass. The right side of the carcass was weighed, fabricated using the procedures described by NAMP (1992), and weights recorded for carcass cutting yields. Trimmed, boneless, wholesale cuts were obtained and the weights were recorded.

Meat quality measurements. Longissimus pH was measured at 45 min and 24 h postmortem. Minolta color (L*, a*, and b*) was measured on the cut surface of the longissimus and the semimembranosus using a CR-300 Minolta Chromameter (Minolta Camera Co., Ltd., Japan). Loin chops (2.5 cm thick) were obtained from the longissimus immediately posterior to the last rib from the left side of the carcass. One chop was weighed, placed in a Whirl-pak bag, and suspended in a 4°C cooler for 24 h then reweighed and drip loss recorded and an additional chop was recovered and used for glycolytic potential determination by enzymatic assay.

Glycolytic potential determination. Glycolytic potential on the longissimus chop sample was determined by enzymatic assay. Metabolite concentrations for glucose, glucose-6-phosphate, glycogen, and lactate were determined. Glycolytic potential values (in μmol lactate equivalents / g muscle) were calculated using the formula described by Monin and Sellier (1985) as follows:

Glycolytic potential = 2 ([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate].

Results

A frequency distribution for glycolytic potential of the animals used for this study is presented in Figure 1. Only animals with glycolytic potential lower than 160 umol/g were included in the analysis of relationships between glycolytic potential and economically important traits.

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Relationships between glycolytic potential and average daily gain (Figure 2) and carcass traits such as tenth rib backfat (Figure 3) and percentage lean cut yield (Figure 4) were very weak suggesting little association between glycolytic potential and growth and carcass traits. Glycolytic potential showed unfavorable relationships with the ultimate pH and Minolta L* values for the ham (semimembranosus; Figures 5 and 6, respectively) and loin (longissimus; Figures 7 and 8, respectively); and with longissimus drip loss (Figure 9).

Conclusions

1. The results of this study suggest that the association between glycolytic potential and growth rate and carcass characteristics is very weak.

2. The unfavorable relationships between glycolytic potential and meat quality traits suggest that reducing glycolytic potential would improve meat quality within a population with normal levels of glycolytic potential.

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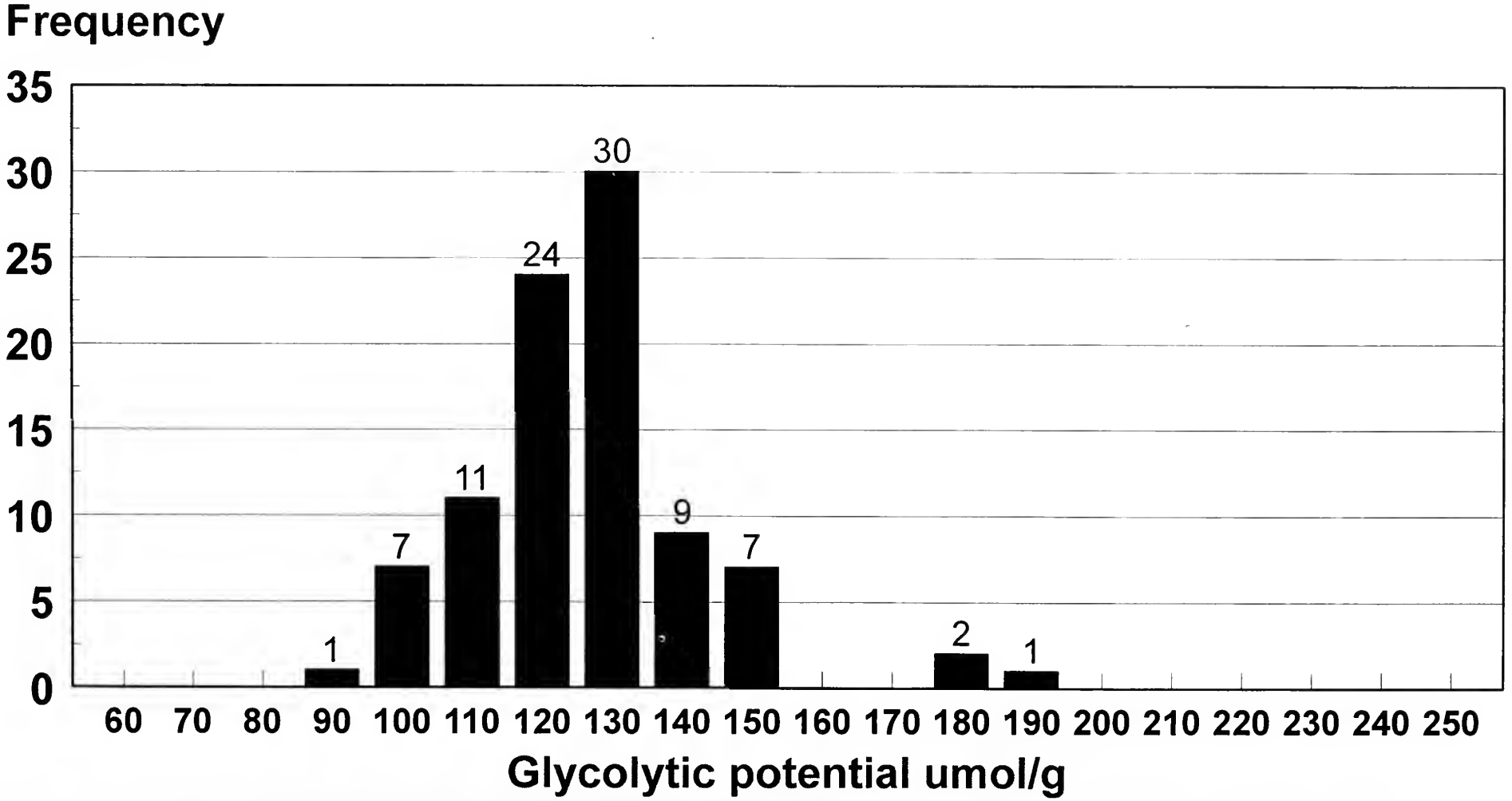
Figure 1. Distribution of glycolytic potential.



Only animals with glycolytic potential < 160 umol/g were used in the analysis (n=89)

Figure 2. Linear regression of glycolytic potential and average daily gain (kg) within a population with low glycolytic potential.

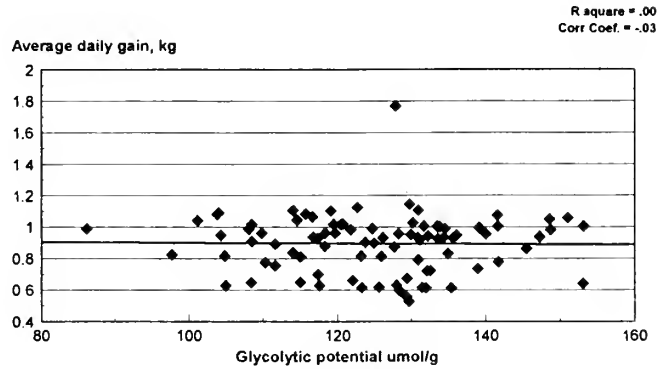


Figure 3. Linear regression of glycolytic potential and tenth rib backfat (cm) within a population with low glycolytic potential.

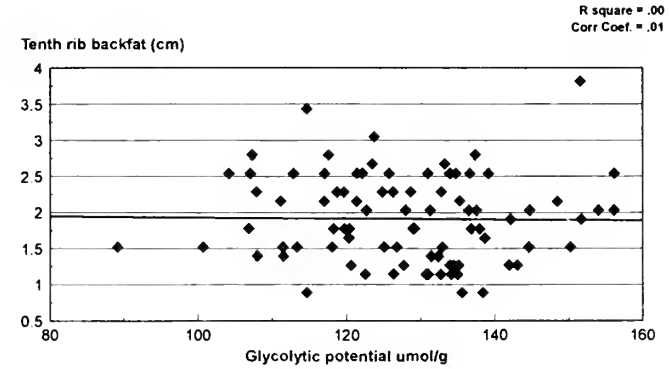


Figure 4. Linear regression of glycolytic potential and lean cut yield of side weight within a population with low glycolytic potential.

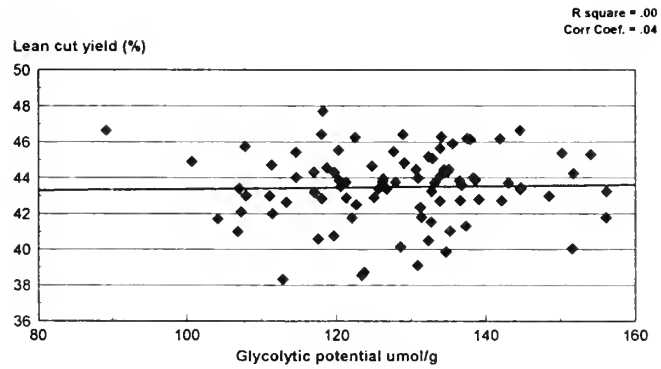


Figure 5. Linear regression of glycolytic potential and semimembranosus ultimate pH within a population with low glycolytic potential.

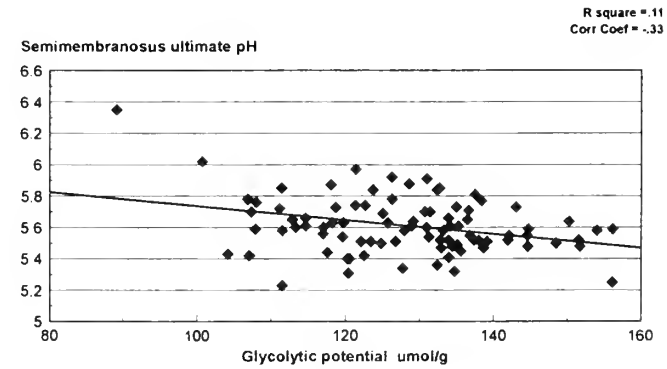


Figure 6. Linear regression of glycolytic potential and semimembranosus minolta L* values within a population with low glycolytic potential.

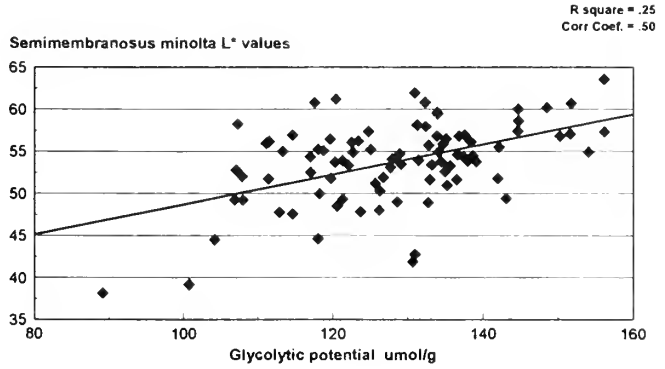


Figure 7. Linear regression of glycolytic potential and longissimus ultimate pH within a population with low glycolytic potential.

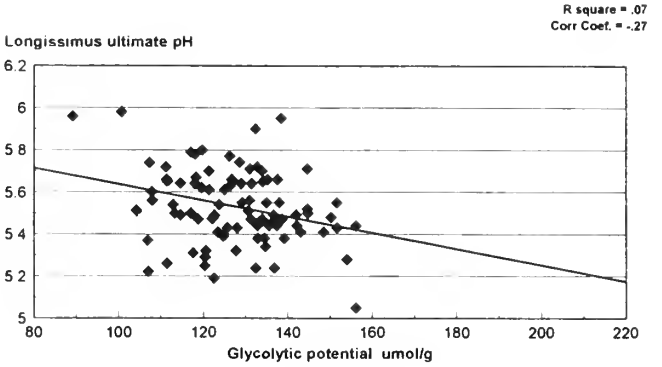


Figure 8. Linear regression of glycolytic potential and longissimus minolta L* values within a population with low glycolytic potential.

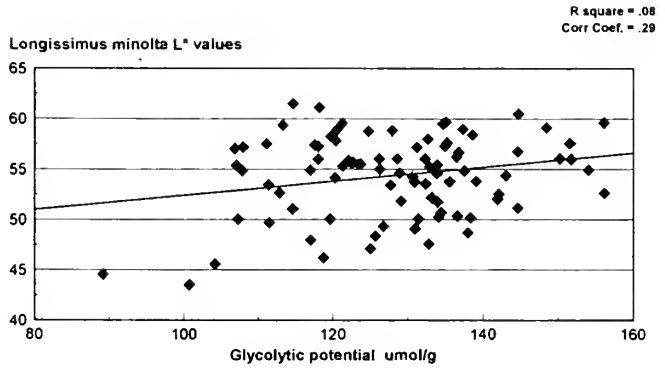
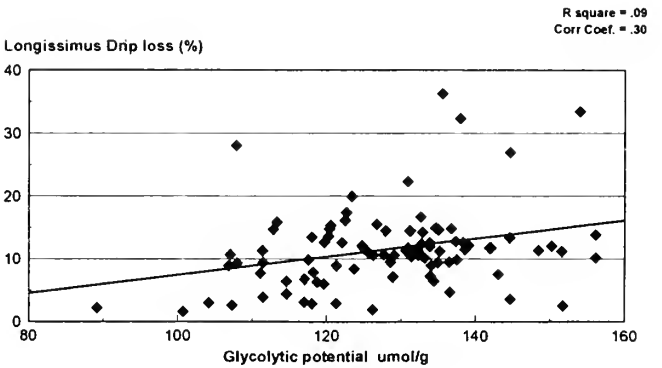


Figure 9. Linear regression of glycolytic potential and longissimus drip loss (%) within a population with low glycolytic potential.



Influence of Dietary Selenium Source on Growth Performance, and Carcass and Meat Quality Characteristics in Swine

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Introduction

Increasing emphasis is being focused on pig meat quality and, particularly, on trying to identify techniques to improve the water holding capacity of pork and pork products. Approaches that have been attempted include the feeding of antioxidants such as vitamin E and elements such as selenium, which act to prevent oxidative damage to cell membranes and improve their integrity, thereby reducing moisture loss from the cell. Selenium in combination with the enzyme glutathione peroxidase (GSH-Px), in conjunction with vitamin E and other antioxidative agents, is capable of reducing the destructive effects on living cells of peroxidative reactions (Pehrson, 1993).

Typically, organic sources of selenium, such as sodium selenite, are most commonly used in feed supplements for swine. However, organic sources, including selenium yeasts, are also available and there is evidence that the use of such products, in comparison with sodium selenite, has resulted in a reduction in muscle drip loss in poultry (Edens, 1996) and there have been unsubstantiated claims of similar effects in swine. The objective of this study was to evaluate the impact of supplying selenium either in the form of sodium selenite or as a selenium-enriched yeast on growth, carcass and meat quality characteristics in swine.

Experimental Approach

The study was conducted at the Swine Research Center at the University of Illinois. Two sources of supplementary dietary selenium were compared, namely sodium selenite and a selenium-enriched product (Sel-Plex 50; Alltech, Nicholasville, KY) which has a high selenium content (1,000 ppm) and contains various organic seleno amino acid analogs, principally selenomethionine (Kelly and Powers, 1995). Premixes containing either sodium selenite or Sel-Plex 50 were included at 0.15% of the diet and provided 0.3 ppm of added selenium. Diets were based on corn and soybean meal and a three-phase dietary program was used. The first phase diet was fed between 20 to 40 kg and was formulated to supply 19 % crude protein, 1.12% lysine, and 3390 Kcal/kg ME. The second phase diet was fed between 40 to 80 kg and was formulated to supply 18.2 % crude protein, 1.05% lysine and 3380 Kcal/kg ME. The third phase diet was fed between 80 to 110 kg and was formulated to supply 16.5 % crude protein, 0.9% lysine and 3390 Kcal/kg ME.

The animals used were Halothane carrier gilts supplied by a commercial breeding company (PIC line 406). A total of 48 pigs were allocated to two treatment groups of 24 pigs, on the basis of weight. The study was carried out between live weights of approximately 22 to 110 kg. During the study, pigs were housed in pens of four in a controlled environment grow-finish house which had partially slated flooring. Animals were given ad libitum access to feed

from a two-hole feeder and water was continuously available via a nipple drinker in each pen.

Pigs were weighed every fourteen days in the early part of the study and weekly as they approached the designated slaughter weight. Feed additions were recorded and feeder weights taken at each weighing. After completing the test period, pigs remained in their test groups on full feed until they were dispatched from the farm on the morning prior to slaughter.

Forty-two (n=42) pigs were transported to a slaughter facility located approximately 150 miles from the University where they were held approximately 16 h prior to slaughter. Animals were mixed with those from other groups during transport and in the lairage where they were held overnight without food but with access to water. Slaughtering was carried out using commercial procedures. Carcasses were split down the midline, and placed in a chiller (held at 4° C) approximately 75 minutes postmortem where they were held overnight.

At 24 h postmortem, cold carcass weights were recorded, and carcass measurements were obtained on the left side of each carcass. These measurements included: carcass length (measured from the cranial tip of the aitch bone to the cranial edge of the first rib adjacent to the thoracic vertebra), midline fat measurements (opposite the first rib, last rib, and last lumbar vertebra), fat depth in the middle of the loin eye and loin eye area at the tenth rib.

Longissimus pH was evaluated at 48-h postmortem. A muscle sample (approximately 3 g) was obtained at the tenth rib and homogenized in 10 ml of iodacetate. The pH of the sample was obtained using an Orion model 720A pH meter fitted with a Ross sure flow 81-72 electrode (Orion Research, Boston, MA). Longissimus color, firmness, and marbling scores were taken using the procedures described by NPPC (1994). Longissimus color at the last rib (L*, a*,b* values) was evaluated at 48-h postmortem using a Hunter Chromameter. Three longissimus chops (2.5 cm thick) were obtained from the left side of the carcass immediately posterior to the tenth rib. One chop was weighed, placed in a Whirl-pak bag, and suspended in a 4 °C cooler for 24 h then reweighed and drip loss recorded. One chop was trimmed of all fat, homogenized, then placed in a Whirl-pak bag and frozen (-20° C) for subsequent chemical analysis. Fat and water contents were determined using the procedures described by Novakoski et al. (1989). All samples were oven dried to constant weight (110° C for 24 to 48 h). Fat was extracted using chloroform:methanol mixture (4:1). The remaining chop was used for water holding capacity determination which was carried out using an approach that was adapted from Method 18 (Mass, 1989; Seman 1991). Duplicate 15 g homogenized samples were weighed into 50 ml centrifuge tubes and 25 ml of a hydrating solution (3.5 % NaCl, .04M Na_2HPO_4) was added. The mixture was allowed to stand for 30 min (25° C) before being centrifuged at 1000 x g for 10 min. The tubes were decanted of free moisture and a post-centrifuge weight was recorded and used to determine the percentage water holding capacity. Following decanting, the samples were cooked in a water bath at 80° C for 30 min. The cooked-out water and fat were decanted, and a cooked weight was recorded and utilized to determine the percentage cooking loss. Water holding capacity was divided by the protein percentage to correct for differences in protein content. Protein content was determined on duplicate samples using the Kejdahl procedure.

Data were analyzed using the PROC GLM procedure of SAS (1985) with the model used including the effect of dietary selenium source. For the growth data, the pen was considered as the experimental unit, with the individual animal being used as the experimental unit for the carcass and meat quality data.

Results

The least square means for selenium sources for growth performance, slaughter and carcass measurements, and meat quality measurements are presented in Table 1, Table 2, and Table 3, respectively. Growth rates, feed intakes, and gain:feed ratio did not differ between the two sources of dietary selenium (Table 1). Dressing percentage, carcass length, and first, tenth and last rib fat depths were similar for the two selenium sources (Table 2); however, pigs fed the diets containing the selenium-enriched yeast produced carcasses with lower backfat depths at the last lumbar vertebra ($P<.05$) and larger loin eye areas ($P<.05$) compared to animals consuming the inorganic source of dietary selenium (Table 2). There were no differences in meat quality or water holding capacity measurements among the two dietary selenium sources.

Conclusions

The results of this study, therefore, suggest that utilizing selenium yeast as a source of supplemental selenium in pig diets has no effect on the quality or water holding capacity of pig meat. However, the positive effects of the organic selenium source on backfat thickness and loin eye area warrant further study.

Acknowledgments

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Table 3. Least squares means for inorganic and organic selenium source for meat quality measurements taken at the longissimus.

Traits	Inorganic Selenium	Organic Selenium	Avg SE	P value
24 hr. pH	5.37	5.38	.025	.80
Color[a]	1.90	2.04	.159	.53
Firmness[a]	2.04	1.95	.146	.68
Marbling[a]	1.43	1.46	.119	.84
Hunter L*	56.07	56.26	.856	.87
Hunter a*	5.76	5.92	.247	.65
Hunter b*	16.50	16.44	.185	.81
Drip Loss, %	2.56	2.74	.307	.68
Cooking Loss, %	27.31	28.71	.659	.15
Water holding capacity, %	66.99	66.46	1.557	.80
Water holding capacity, % (equal protein basis)	3.04	2.90	.106	.35
Proximate Composition, %				
Protein	22.31	22.90	.368	.26
Water	75.44	75.38	.176	.79
Fat	1.81	1.48	.148	.12

[a] Subjective color, firmness, and marbling scores where 1=pale, soft and devoid of marbling and 5=dark, firm and moderately abundant marbling

Table 1. Least squares means for inorganic and organic selenium sources for growth performance.

Traits	Inorganic Selenium	Organic Selenium	Avg. SE	P value
Weight. at start of test, kg	21.80	22.39	1.155	.72
Weight at end of test, kg	108.83	108.79	1.734	.98
Average daily feed intake, kg	2.50	2.46	.046	.55
Average daily gain, kg	.89	.89	.008	.61
Gain:feed	.35	.36	.004	.63

Table 2. Least squares means for inorganic and organic selenium source for slaughter and carcass measurements.

Items	Inorganic Selenium	Organic Selenium	Avg SE	P value
Cold carcass weight, kg	78.83	79.51	.299	.11
Dressing percentage	71.24	71.83	.270	.12
Carcass Length, cm	79.92	80.19	.318	.55
Backfat depths, mm				
First rib	328	330	9.7	.86
Last rib fat	203	192	6.9	.27
Last lumbar fat	139	116	7.7	.04
Tenth rib fat[a]	139	144	7.1	.60
Loin eye area, cm^2	40.37	42.82	.795	.03

[a] measured off the midline, in middle of loin eye

The influence of time of feeding of amino-acid deficient diets on the intramuscular fat content of pork.

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Introduction

The substantial decline in carcass fat levels that has occurred in pigs has been accompanied by a reduction in the amount of intramuscular fat, or marbling. The relationship between intramuscular fat and pork eating quality is not clearly established. However, there are reports that tenderness and juiciness improves with increasing intramuscular fat (Meat and Livestock Commission, 1992; Castell et al., 1994) and of a threshold or minimum level for marbling that is required to ensure good eating quality (Bejerholm and Barton-Gade, 1986; De Vol et al., 1988). The most commonly adopted approach to meeting these specification is the use of breeds with high intramuscular fat levels, particularly the Duroc and there is evidence of an advantage for this breed in terms of both marbling fat and eating quality (Ellis et al., 1996). Another approach that would also allow the production of higher, and perhaps targeted, levels of intramuscular fat to be produced is by manipulating the nutrition of the pigs. There are a number of reports of substantial increases in intramuscular fat levels resulting from the feeding of protein deficient diets throughout the growing and finishing periods (Castell et al., 1994; Kerr et al., 1995; Goerl et al., 1995; Blanchard et al., 1997). However, these studies also reported high carcass fat levels and low feed efficiency from the use of protein deficient diets and such an approach to increasing levels of intramuscular fat is likely to be uneconomic in most situations. However, fat deposition rates increase with the weight of the animal and Cisneros et al. (1996) reported substantial increases in fat content of the longissimus dorsi as a result of feeding amino acid-deficient diets during the last 3 and 5 weeks of the finishing period. The objective of this study was to establish the degree of dietary amino acid deficiency and the time of feeding of amino acid-deficient diets before slaughter that is required to increase intramuscular fat levels.

Experimental Procedures

Three studies were carried out for periods of one, three, or five weeks prior to slaughter, respectively. Within each study, four dietary treatments were used, involving different levels of amino acids (0.40 v. 0.48 v. 0.56 v. 0.64 % lysine) at the same crude protein content (Table 1). The diet with the highest lysine level was formulated to meet the requirements of finishing pigs of the genotype used in this study (Hahn and Baker, 1995; Hahn et al., 1995). A basal diet containing 10.0% crude protein per kg was formulated using corn, soybean meal, soybean oil and mineral and vitamin supplements. This diet was supplemented with synthetic amino acids (L-lysine, L-threonine, DL-methionine, and L-tryptophan) and/or starch to achieve the required nutrient composition in the four diets (Table 1)

Seventy-two gilts from a commercial hybrid line were used in this study. The live weights at the start of the study period were 107.9 (SD 2.20), 97.0 (SD 1.91) and 67.34 (SD 2.04) kg for the one-, three- and five-week studies, respectively. Within each study, gilts were formed into

outcome groups of four animals, on the basis of live weight and litter of origin. Animals were randomly allocated from within outcome group to one of the four dietary treatments. The study was carried out at the Swine Research Center of the University of Illinois. Animals were individually housed in a controlled environment building where the temperature was set at 18°C throughout the study. Pens (dimensions 2m x 1.5m) were equipped with a nipple drinker and an ad libitum feed hopper. Pigs had continuous access to food and water. Fresh food was added to the feeder on a daily basis, with refusals being collected and weighed on a weekly basis. Animals were weighed at the start of the study and once per week thereafter and the test ended after a 7, 21 or 35 day period, respectively.

Animals were transported to the Meat Science Laboratory, University of Illinois on the afternoon of the day they completed the test period and were slaughtered the following morning after a period in lairage of approximately 18 h. Slaughter and carcass dressing were carried out using standard procedures. The head, kidneys and flare fat were removed and the carcass was split down the mid line. A hot carcass weight (kg) was taken approximately 1-h post mortem at which time carcasses were placed in a chiller (at 4^0C) where they were held overnight.

At 24-h post mortem, fat depth over the center of the loin eye at the 10^{th} rib (mm) and loin eye area at the 10^{th} rib (cm^2) were measured on the left side of the carcass. Longissimus dorsi muscle color (L* value) was measured using a Hunter Chromameter. Muscle samples for chemical analysis were obtained from the longissimus dorsi immediately posterior to the 10^{th} rib and opposite the 3rd/4th lumbar vertebra. A 2-cm thick section of muscle was taken and trimmed of epimysium and connective tissue prior to homogenizing. Proximate analysis procedures for fat and moisture content were conducted in duplicate on the homogenized muscle samples using the procedures described by Novakofski et al. (1986). Fat determinations were carried out using extraction with an azaeotropic mixture of warm chloroform and methanol (4:1).

For each trial, statistical analysis was carried out using the General Linear Models procedures of Statistical Analysis Systems (1988) with the model used accounting for the effects of amino acid level.

Results

The effects of dietary lysine level on growth performance is summarized in Table 2. Pigs on the five-week study were lighter at the start and end of the test period than those on the other studies. For the one- and three-week study periods, there was no effect of lysine level on feed intake, daily gain, or gain:feed ratio. For the five-week study, pigs on the lowest lysine level grew more slowly ($P<0.001$) than those on the other treatments (Table 2). Gain:feed ratio was highest for pigs fed the 6.4 g/kg lysine diet, lowest for those fed the 0.40 % lysine diet, and intermediate for the other diets.

The slaughter, carcass and meat quality characteristics and longissimus fat content of pigs on the different lysine levels are summarized in Table 3. In the one-week study, the only dietary treatment effect was for tenth rib backfat thickness which was higher for pigs on the 0.64% lysine diet than for the other treatments. For the three-week study, there was no effect of dietary

lysine level on any of the variables (Table 3). For the five-week study, loin eye area tended to increase with dietary lysine level. In addition, there was a response in intramuscular fat to lysine deficiency with longissimus fat contents being greater for the 0.48% lysine level than for higher or lower levels (Table 3).

Conclusions

This study suggests that periods of feeding of amino acid deficient diets of approximately 5 weeks are required to produce an increase in intramuscular fat levels and that there is an optimum lysine level at which the maximum response in intramuscular is achieved. Feeding diets with lysine levels above or below this optimum level will not be as effective at increasing intramuscular fat levels. This suggests that to achieve substantial increases in marbling using protein deficient diets will require a relatively precise approach, both in terms of the degree of deficiency used and the duration of the feeding period. In addition, the results for loin eye area suggest that increases in marbling fat are likely to be accompanied by a reduction in carcass lean content.

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Table 1. Composition of diets (as-fed basis)

Item	Lysine level (%) 0.40	0.48	0.56	0.64
Ingredients (%)				
Maize	88.6	88.6	88.6	88.6
Soyabean meal	6.28	6,28	6.28	6.28
Soyabean oil	2.06	2.06	2.06	2.06
Starch	0	0.057	0.131	0.208
L-Lysine	0	0.10	0.20	0.30
L-Tryptophan	0	0	0.012	0.026
L-Threonine	0	0	0.045	0.101
Glutamic acid	0.635	0.478	0.247	0
Di-calcium phosphate	1.05	1.05	1.05	1.05
Limestone	0.82	0.82	.0.82	0.82
Trace-mineral premix[a]	0.36	0.36	0.36	0.36
Vitamin premix[b]	0.21	0.21	0.21	0.21
Composition (% as fed)				
Crude protein [c]	10.00	10.25	9.94	9.94
Total lysine[c]	0.43	0.51	0.61	0.65
Total threonine[c]	0.39	0.39	0.43	0.49
Total isoleucine[c]	0.38	0.37	0.39	0.38

[a] Each kilogram of trace-mineral mix contained the following: Se 85.7 mg, I 100 mg, Cu 2.3 g, Mn 5.7g, Fe 25.7g, Zn 28.6g, NaCl 855g.

[b] Each kilogram of vitamin mix contained the following: vitamin A 3,300,00 IU, vitamin D_3 330,000 IU, vitamin E 44,000 IU, vitamin K 2.2g, vitamin B_{12} 27.9 mg, riboflavin 4.4 mg; d-pantothenic acid 12.2 g; choline chloride 165g and roughage products to lkg.

[c] Calculated.

Table 2. Growth performance of pigs fed with graded levels of lysine for one, three or five weeks periods before slaughtering

	Lysine level (%)					
Study period/Trait	0.40	0.48	0.56	0.64	SEM	Significance
One week:						
Initial weight, kg	106.6	105.3	108.2	111.6	1.97	NS
Final weight, kg	112.8	111.5	114.7	119.0	2.44	NS
Average daily gain, g	0.89	0.87	0.92	1.06	0.160	NS
Average daily feed intake, g	3.20	3.13	3.58	3.60	0.535	NS
Average daily lysine intake, g	13.6	15.9	21.9	23.6	2.89	NS
Gain:feed	0.32	0.37	0.28	0.31	0.074	NS
Three week:						
Initial weight, kg	97.7	98.6	96.4	95.2	1.48	NS
Final weight, kg	114.4	114.8	115.1	115.4	2.29	NS
Average daily gain, g	0.79	0.77	0.89	0.96	0.058	NS
Average daily feed intake, kg	3.12	3.12	3.25	3.22	0.192	NS
Average daily lysine intake, g	13.3^{a}	15.9^{a}	19.9^{b}	21.1^{b}	1.13	***
Gain:feed	0.25	0.25	0.29	0.30	0.021	NS
Five week:						
Initial weight, kg	67.2	67.0	68.0	67.4	1.71	NS
Final weight, kg	89.1^{a}	96.9^{b}	97.0^{b}	96.7^{b}	1.97	*
Average daily gain, g	0.63^{a}	0.85^{b}	0.83^{b}	0.84^{b}	0.029	***
Average daily feed intake, kg	2.80	3.02	2.94	2.62	0.130	NS
Average daily lysine intake, g	11.9^{a}	15.4^{b}	18.0^{b}	17.1^{bc}	0.63	***
Gain:feed	0.23^{a}	0.28^{b}	0.28^{b}	0.32^{c}	0.011	***

[a,bc,] Means within the same row with different superscripts differ ($P<0.05$).

NS, *, *** = not statistically significant, $P<.05$, $P<.001$, respectively.

ble 3. Carcass and meat quality characteristics and longissimus fat content (intramuscular fat) of pigs fed th graded levels of lysine for one, three or five weeks before slaughter

	Lysine level (%)					
udy Period/Trait	0.40	0.48	0.56	0.64	SEM	Significance
ne week:						
Slaughter weight, kg	109.60	108.32	111.68	115.54	2.003	NS
Hot carcass weight, kg	81.08	79.49	82.51	85.35	0.699	NS
Dressing percentage (hot)	73.92	73.37	73.89	73.88	0.455	NS
Tenth rib backfat, cm	1.84[a]	1.71[a]	1.74[a]	2.41[b]	0.169	*
Loin eye area, cm^2	37.10	35.43	36.83	34.19	1.436	NS
Drip loss, %	8.53	8.46	8.16	8.12	0.880	NS
Hunter color, L*	52.39	54.49	53.19	55.03	1.382	NS
Longissimus fat content, %						
10^{th} rib	3.22	3.16	3.15	2.90	0.268	NS
3rd-4th lumbar	4.12	3.14	3.44	3.37	0.567	NS
ree week:						
Slaughter weight, kg	110.55	111.46	111.42	112.10	2.218	NS
Hot carcass weight, kg	82.51	82.40	82.55	82.97	1.686	NS
Dressing percentage (hot)	74.66	73.93	74.08	74.01	0.342	NS
Tenth rib backfat, cm	1.82	1.69	1.74	1.69	0.144	NS
Loin eye area, cm^2	37.26	40.32	38.71	39.19	1.559	NS
Drip loss, %	6.06	5.58	7.02	6.48	1.059	NS
Hunter color, L*	52.67	51.15	53.96	52.18	1.122	NS
Longissimus fat content, %						
10^{th} rib	3.08	2.81	2.82	3.05	0.245	NS
3rd-4th lumbar	4.73	3.21	4.50	4.69	0.453	NS
ve week:						
Slaughter weight, kg	87.08	93.26	94.51	93.55	2.491	NS
Hot carcass weight, kg	63.30	68.18	70.48	69.69	2.054	NS
Dressing percentage (hot)	72.65	73.14	74.54	74.44	0.691	NS
Tenth rib backfat, cm	1.74	1.69	1.57	1.42	0.113	NS
Loin eye area, cm^2	28.06[a]	30.48[ab]	33.98[c]	33.42[bc]	1.022	**
Drip loss, %	5.43	4.37	6.04	6.79	0.805	NS
Hunter color, L*	52.47	54.73	54.73	53.54	1.003	NS
Longissimus fat content, %						
10^{th} rib	3.34[ab]	3.97[b]	3.46[ab]	2.82[a]	0.222	**
3rd-4th lumbar	3.46[a]	5.06[b]	3.34[a]	2.83[b]	0.429	**

Means within the same row with different superscripts differ (P<0.05).
S, *, ** = not statistically significant, P<.05, P<.01, respectively.

The influence of constant versus fluctuating daily feed intake on the growth performance of growing and finishing pigs.

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Introduction

Previous research at this center based on computerized feed intake recording equipment has shown considerable variation in the feeding patterns of growing and finishing pigs (Hyun, 1997). In particular, the variation in feeding behavior among animals of the same genotype, sex and weight fed the same diet and housed in the same pen appears to be huge. For example, Hyun et al. (1997) showed that the average number visits to the feeder for individual pigs ranged from 6 to 26 per day. In addition, the day to day variation in feed intake for individual pigs also appears to be considerable and can be as high as 100% or even greater on consecutive days for some animals Hyun, 1997). However, there has been little research to evaluate the impact of day-to-day variation in feed intake levels on growth performance. This study was carried out to investigate the impact of daily variation in feeding level on growth performance in two weights of pigs.

Experimental Approach

The study was carried out a 3 x 2 factorial design with three feeding regimens and two weights of pig. The feeding regimens were as follows:

1. Ad libitum (AL).
2. Restrict fed to 95% of ad libitum intake, with the feeding level being kept constant from day to day (95AL-FIXED).
3. Restrict fed to 95% of ad libitum intake, with the feeding level being varied from plus 15% to minus 15% of ad libitum intake on consecutive days (95AL-VAR).

The feed intake of the pigs on the AL treatment was measured on a daily basis and this data was used to calculate the feeding level (95% of ad libitum intake) for the other two feeding regimes.

The study was carried out over a 42-day period with the two weight ranges studied being from from 44 to 80 kg and 84 to 123 kg, respectively. A total of 72 barrows that were the progeny of PIC line 326 sires and C15 dams were used in the study, giving 12 pigs per feeding regime x weight subclass. Pigs were housed in individual pens which had fully slatted floors and contained an ad libitum feed hopper and nipple water drinker. The diet used over the lighter weight range contained 18 % crude protein, with that used for the heavier animals having 16% crude protein. Animals were weighed once per week to monitor growth rate and feed intake was recorded on a weekly basis. Pigs were ultrasonically scanned at the start and end of the study.

Longitudinal scans were taken immediately anterior to the last rib and average backfat thickness and loin eye depth were automatically measured on the scans.

Results

The results for the impact of feeding regimen on growth performance and ultrasonically measured carcass characteristics for growing and finishing pigs is summarized in Table 1. As anticipated, the performance levels and carcass measurements were substantially different for the lighter and heavier weight ranges studied. However, there were no statistically significant differences between the feeding regimes for growth and carcass traits for either weight range. Pigs fed ad libitum did have higher feed intakes and growth rates than those on the other two regimens. In addition, for growing pigs fed at 95% of the ad libitum treatment, there was a numerical trend for pigs on the variable feeding regimen to grower faster than those on the fixed regimen; however for finishing pigs, the reverse trend was seen.

Conclusions

The results of this study suggests that fluctuating daily feed intakes within the range of 15% either side of the mean do not significantly impact animal performance or carcass leanness. The impact of wider daily fluctuations in feed intake, which are common in group-housed pigs on automated feed intake equipment and also are likely to occur under commercial conditions, is uncertain and needs to be studied.

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Table 1. The effect of feeding regimen on growth performance and ultrasound measurements for growing and finishing pigs										
Weight:	Growing pigs					Finishing pigs				
	AL	95AL-FIXED	95AL-VAR	Avg. SE	Sig.	AL	95AL-FIXED	95AL-VAR	Avg. SE	Sig.
Initial weight, kg	43.48	43.71	43.92			83.93	83.57	83.55		
Final weight, kg	82.64	78.30	80.98			125.84	122.64	120.59		
Average daily gain, kg	0.897	0.798	0.861	0.044	NS	1.050	0.982	0.944	0.054	NS
Average daily feed intake, kg	2.598	2.410	2.397	0.116	NS	3.030	2.821	2.833	0.143	NS
Feed:gain (kg/kg)	3.45	3.35	3.64	0.133	NS	3.57	3.55	3.43	0.165	NS
Final backfat thickness (cm)	1.302	1.246	1.269	0.110	NS	1.67	1.78	1.65	0.124	NS
Final loin eye depth (cm)	3.95	4.00	4.21	0.156	NS	5.37	5.01	5.09	0.175	NS

The effect of feeding level and physiological state on total flow and amino acid composition of endogenous protein in swine

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Abstract

An experiment was conducted with the objective of investigating the effects of BW, feed intake, and the physiological condition of the animal on the loss and amino acid composition of endogenous protein in swine. The total endogenous protein loss at the distal ileum of 110 kg growing pigs, lactating sows, and gestating sows, given free access to feed, was 12.4 g, 9.4 g, and 11.2 g/kg DMI, respectively. These values were not different ($P > .10$). However, when gestating sows were fed only 2 kg/d, 17.8 g of endogenous protein were lost per kg DMI, which was higher ($P < .05$) than for any of the other groups. Likewise, restricted-fed gestating sows had an amino acid composition of endogenous protein that was significantly different from that of the other groups. The results from the experiment showed that age, BW, and the physiological condition of the animal have little or no effect on the amount of endogenous protein and amino acids lost at the distal ileum of pigs if calculated relative to DMI. However, daily feed intake had a significant effect on endogenous protein losses in sows as well as on amino acid composition of endogenous protein.

Introduction

Historically, endogenous protein has been defined as the protein recovered in fecal material from animals fed a protein-free diet (Mitchell, 1924). Because the amino acid composition of protein is altered by the microbes in the hindgut, it is now accepted that protein and amino acid losses should be determined at the distal ileum rather than in fecal output (Sauer and Ozimek, 1986; Sauer and de Lange, 1992).

Nutrient digestibilities and endogenous losses are usually determined in weanling or growing pigs. Subsequently, these numbers are applied to all categories of swine, and it has been proposed that the amount of endogenous losses as well as the amino acid composition of endogenous protein is constant regardless of the live weight of the animal (Boisen and Moughan, 1996). However, the hypothesis that values for endogenous losses obtained in growing pigs also can be applied to gestating and lactating sows has never been tested. Likewise, it is not documented that the amino acid composition of endogenous protein is constant, regardless of the physiological state or the feed intake of the animal. The objective of the present work was to test these hypotheses.

Materials and methods

Ten growing barrows (average BW: 110 ± 3.9 kg) arising from the matings of Camborough 15 sows to PIC line 326 boars (PIC, Franklin, KY) were used to measure endogenous losses for growing pigs. Five adult multiparous sows (Camborough 15, PIC, Franklin, KY) were used to determine endogenous losses for sows under different physiological conditions and subjected to different feeding regimes. All animals were prepared with a T-cannula surgically installed in the distal ileum. A protein-free diet containing .25% chromium oxide as an indigestible marker was formulated (Table 1).

The experiment consisted of four collection periods. The growing pigs were collected once, and the sows were collected in lactation and twice in the following gestation period. The growing pigs and the lactating sows were allowed ad libitum access to the experimental diet. Gestating sows were restricted to 2 kg feed/d in the first collection period, but the diet was fed to appetite during the second collection period. However, only four sows were used in this last period. All animals were fed the experimental diet for 7 d with digesta being collected over two 12 h periods on d 6 and 7.

Data for feed intake were summarized at the end of each collection period, and average daily feed intake was calculated for each pig and period on a total basis as well as in relation to the metabolic body weight ($kg^{0.75}$) of the animals. The endogenous flow of each amino acid was calculated for each animal and period as mg lost/kg DMI. The amino acid composition of endogenous protein was calculated for each animal group by expressing each amino acid as a percentage of total endogenous protein.

Results were subjected to analysis of variance using the Proc GLM procedure of SAS (SAS, 1995), and treatment means were separated using the least square difference test.

Results

Animals remained healthy throughout the experiment and readily consumed their diets (Table 2). Growing pigs consumed an average of 2.67 kg/d of feed, but lactating sows and gestating sows given free access to feed consumed more ($P < .05$), 5.05 kg and 4.35 kg/d, respectively. However, if feed intake was calculated in relation to metabolic BW of the animals, there were no differences in daily feed intake ($P > .05$) among these three groups. Restricted fed gestating sows were allowed to eat only 2 kg of feed/d, which was significantly less ($P < .05$) than what sows given free access to feed consumed on a daily basis as well as in relation to metabolic BW. All animals lost weight during the week they were fed the protein-free diet (Table 2). Lactating sows lost more weight ($P < .05$) than did any of the other groups during this week.

Endogenous losses of amino acids and protein for each animal group are presented in Table 3. The endogenous protein loss of lactating sows was numerically lower, but not significantly different ($P = .11$) from that of growing pigs or gestating sows given free access to feed. However, lactating sows had the numerically lowest loss of all amino acids except for tryptophan and glycine compared to the other groups, but not all of these differences were significant. The restricted-fed gestating sows had a greater ($P < .05$) loss of endogenous protein than any of the other groups. Likewise, the losses of all amino acids were numerically higher in restricted-fed sows compared to the other groups, significantly so for the loss of arginine, histidine, phenylalanine, glycine, proline, and serine ($P < .05$).

The amino acid composition of endogenous protein in lactating sows and gestating sows allowed ad libitum access to the diet was not different ($P > .05$) from the composition of endogenous protein in growing pigs, except for glycine in lactating sows (Table 4). The amino acid composition of endogenous protein in gestating sows fed only 2 kg/d was significantly different from that in growing pigs for all amino acids except arginine, histidine, glycine, and serine. Furthermore, these sows had a lower ($P < .05$) amount of indispensable amino acids and a higher ($P < 0.5$) amount of dispensable amino acids in endogenous protein than the other groups. In particular, the loss of proline in sows restricted in their feed intake was higher ($P < .05$) than in any of the other groups.

Discussion

The values for endogenous losses of protein and amino acids for growing pigs are in agreement with those previously reported in studies in which a protein-free diet had been fed (e.g., de Lange et al., 1989a, 1989b; Wang and Fuller, 1989; Leterme et al., 1996). Furthermore, the amino acid composition of endogenous protein from growing pigs in this study parallels previous estimates (Wünsche et al., 1987).

The values for endogenous losses obtained in growing pigs were close to those obtained in sows given free access to feed, although the levels were somewhat lower in lactating sows. However, lactating sows had a significantly higher feed intake than did the growing pigs. Comparing the values obtained in lactating sows with those obtained in gestating sows given free access to feed, it appears that, except for isoleucine, there were no significant differences in the amount of endogenous amino acids recovered at the distal ileum. This observation indicates that the difference between growing pigs and lactating sows is caused by the significant difference in daily feed intake between the two groups of animals rather than by the difference in the physiological condition of the animal.

Furuya and Kaji (1992) compared endogenous losses in 45-kg growing pigs and in 92-kg pigs, and they found no significant differences in endogenous amino acid losses between these two groups of pigs. Our results in growing pigs and sows allowed ad libitum access to feed are in agreement with these observations.

When gestating sows were restricted to 2 kg/d, the endogenous loss of protein was higher ($P < .05$) than that for any of the other groups. The values for arginine, histidine, glycine, serine, and proline were significantly higher ($P < .05$) than those obtained with the other groups, and for all other amino acids the numerically highest values were obtained in gestating sows fed 2 kg/d. This finding indicates that feed intake *per se* has a significant effect on endogenous losses of protein and amino acids if calculated in relation to feed intake. A similar finding has previously been reported (Fuller and Cadenhead, 1991; Furuya and Kaji, 1992; Butts et al., 1993). Therefore, it is important to consider daily feed intake when values for endogenous losses are compared among different experiments.

The composition of endogenous protein was not significantly different ($P > .05$) among the three groups of animals that were fed to appetite (Table 4), but the restricted-fed gestating sows had a significantly different amino acid composition of endogenous protein. This indicates that neither the BW nor the physiological state of the animal affects the amino acid composition of endogenous protein if calculated proportionally to DMI. However, as was the case for the total flow of endogenous protein, the amino acid composition was influenced by the feeding level of the animals.

Implications

The growing pig above 100 kg BW is a good model for estimating the endogenous losses of protein and amino acids at the distal ileum in gestating and lactating sows. Furthermore, the amino acid composition of endogenous protein in sows is not different from that in growing pigs. However, daily feed intake affects the amount of endogenous protein and amino acids lost at the distal ileum as well as the amino acid composition of endogenous protein. Because lactating sows usually have a higher feed intake and gestating sows usually have a lower feed intake than growing pigs, they also have different losses of endogenous protein and amino acids.

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Table 1. Composition (as is) of the experimental diet [a]

Ingredient	g /100 g
Corn starch	81.6
Soybean oil	4.0
Sucrose	5.0
Solka floc[b]	5.0
Dicalcium phosphate	3.2
Limestone	.4
Trace mineral salt [c]	.35
Vitamin premix [d]	.2
Chromium Oxide	.25

[a] .7 g Ca and .6 g P per 100 g diet.
[b] James River, Berlin, NH.
[c] The trace mineral salt provided the following quantities of minerals per 100 g diet: Se, .030 mg; I, .035 mg; Cu, .8 mg; Mn, 2 mg; Fe, 9 mg; Zn, 10 mg; NaCl, 273 mg.
[d] The vitamin premix provided the following quantities of vitamins per kg diet: Vitamin A, 5,250 IU; vitamin D_3, 525 IU; vitamin E, 40 IU; menadione K, 2 mg; vitamin B_{12}, .016 mg; riboflavin, 4 mg; D-pantothenic acid, 11 mg; niacin, 15 mg; choline chloride, 110 mg.

Table 2. Feed intake and average daily gain of animals fed the experimental diet

Item	Pigs	Lactating sows	Gestating Sows		SEM [a]
			Restricted-fed	Ad libitum-fed	
n	10	5	5	4	
BW, kg	111.67 [b]	215.30 [c]	213.00 [c]	234.63 [c]	8.74
BW, $kg^{0.75}$	34.31 [b]	56.11 [c]	55.72 [c]	59.90 [c]	1.76
ADG, g	-132 [b]	-2,800 [c]	-687 [b]	-422 [b]	369
ADFI, kg	2.67 [b]	5.04 [c]	2.0 [b]	4.35 [c]	.28
ADFI, $g/kg^{0.75}$	79 [b]	90 [b]	36 [c]	73 [b]	5.68

[a] Pooled standard error of the mean
[b, c] Means within a row lacking a common superscript are different ($P < .05$)

Table 3. Endogenous losses (mg/kg DMI) of protein and amino acids in growing pigs and sows fed a protein-free diet [a]

Group	Pigs	Lactating sows	Gestating sows		SEM[b]
			Restricted-fed	Ad libitum-fed	
n	10	5	5	4	-
Crude protein	12,437 [c]	9,396 [c]	17,780 [d]	11,218 [c]	1,311
Indispensable amino acids					
Arginine	361 [c]	266 [d]	528 [e]	303 [cd]	32
Histidine	156 [c]	136 [c]	222 [d]	153 [c]	16
Isoleucine	392 [c]	252 [d]	376 [c]	317 [c]	42
Leucine	591 [cd]	446 [c]	644 [d]	498 [cd]	46
Lysine	428 [c]	292 [d]	522 [c]	413 [cd]	46
Methionine	123 [c]	80 [d]	128 [c]	105 [cd]	12
Phenylalanine	341 [cd]	268 [c]	372 [d]	293 [cd]	32
Threonine	527 [cd]	454 [c]	606 [d]	508 [cd]	45
Tryptophan	151	130	162	125	13
Valine	491 [c]	340 [d]	532 [c]	430 [cd]	51
Mean	3,561 [cd]	2,664 [d]	4,092 [c]	3,143 [cd]	332
Dispensable amino acid					
Alanine	591 [c]	406 [d]	650 [c]	510 [cd]	57
Aspartate	878 [c]	604 [d]	1,010 [c]	800 [cd]	85
Cysteine	236 [de]	174 [c]	268 [e]	188 [cd]	16
Glutamate	1,139 [c]	752 [d]	1270 [c]	958 [cd]	111
Glycine	857 [c]	1,020 [c]	1,446 [d]	778 [c]	107
Proline	1,977 [c]	782 [c]	5,044 [d]	1,090 [c]	562
Serine	450 [c]	376 [c]	622 [d]	455 [c]	43
Tyrosine	299 [c]	214 [d]	328 [c]	235 [cd]	28
Mean	6,427 [c]	4,328 [c]	10,638 [d]	5,013 [c]	860
All amino acids	9,988 [c]	6,992 [c]	14,730 [d]	8,155 [c]	1,067

[a] The endogenous loss of protein and each amino acid was calculated as the amino acid or protein concentration in digesta DM multiplied by the ratio between chromium in digesta DM and chromium in feed DM.

[b] Pooled standard error of the mean.

[c, d, e] Means within a row lacking a common superscript are different ($P < .05$).

Table 4. Amino acid composition of endogenous protein in growing pigs and sows [a]

Group	Pigs	Lactating sows	Gestating sows		SEM[b]
			Restricted-fed	Ad libitum-fed	
n	10	5	5	4	
Crude protein	100	100	100	100	
Indispensable amino acids					
Arginine	2.93	2.87	3.00	2.74	.13
Histidine	1.29	1.45	1.25	1.39	.08
Isoleucine	3.19 [c]	2.69 [cd]	2.11 [d]	2.84 [c]	.20
Leucine	4.88 [c]	4.81 [c]	3.64 [d]	4.49 [cd]	.29
Lysine	3.48 [c]	3.12 [cd]	2.96 [d]	3.67 [c]	.18
Methionine	1.01 [c]	.85 [cd]	.72 [d]	.93 [cd]	.06
Phenylalanine	2.82 [c]	2.87 [c]	2.10 [d]	2.62 [cd]	.16
Threonine	4.35 [c]	4.96 [c]	3.42 [d]	4.70 [c]	.27
Tryptophan	1.27 [c]	1.41 [c]	.92 [d]	1.16 [cd]	.08
Valine	3.99 [c]	3.62 [cd]	3.00 [d]	3.93 [c]	.24
Mean	29.21 [c]	28.64 [c]	23.12 [d]	28.46 [c]	1.51
Dispensable amino acids					
Alanine	4.81 [c]	4.34 [c]	3.66 [d]	4.59 [c]	.19
Aspartate	7.20 [c]	6.50 [cd]	5.69 [d]	7.18 [c]	.42
Cysteine	1.94 [c]	1.87 [cd]	1.52 [d]	1.75 [cd]	.10
Glutamate	9.26 [c]	8.06 [cd]	7.12 [d]	8.64 [c]	.42
Glycine	6.85 [c]	11.55 [d]	8.19 [c]	7.13 [c]	.86
Proline	14.38 [c]	7.80 [c]	28.30 [d]	8.88 [c]	3.23
Serine	3.70	4.09	3.49	4.15	.20
Tyrosine	2.46 [c]	2.30 [cd]	1.85 [d]	2.07 [cd]	.14
Mean	50.61 [c]	46.52 [c]	59.83 [d]	44.39 [c]	2.66
All amino acids	79.82 [cd]	75.17 [c]	82.95 [d]	72.85 [c]	2.35

[a] The endogenous loss of each amino acid was calculated as the percentage of total endogenous protein loss.

[b] Pooled standard error of the mean.

[c, d, e] Means within a row lacking a common superscript are different ($P < .05$).

Effects of body weight on total losses and amino acid composition of endogenous protein in growing pigs

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Abstract

An experiment was conducted to investigate the effect of BW on the losses of endogenous protein and the amino acid composition of endogenous protein in growing pigs. A significant ($P < .05$) decrease in endogenous losses calculated as g/kg DMI between 36-kg pigs and 61-kg pigs was observed for protein and amino acids, whereas the estimated endogenous losses of protein and most amino acids in 95-kg pigs were similar to those estimated for 61-kg pigs ($P > .05$). The amino acid composition of endogenous protein changed significantly ($P < .05$) with changing BW of the pigs. The data from this experiment support the hypothesis that the endogenous flow of protein and amino acids at the distal ileum of growing pigs given free access to feed is influenced by the BW of the animals. Hence, a standard value for endogenous losses of amino acids can not be applied to all group of pigs.

Introduction

Estimates for endogenous losses of protein and amino acids have been used to calculate standardized or true digestibility coefficients of amino acids for feed ingredients (Jondreville et al., 1995). In these calculations, only one value for endogenous losses have been used, and it was assumed that this value was representative for all groups of pigs regardless of their BW.

Novel methods for estimating the endogenous flow of protein and amino acids in pigs include the ^{15}N-dilution technique (Souffrant et al., 1981) and the homoarginine technique (Hagemeister and Ebersdobler, 1985). With either of these two methods, only the flow of one or a few amino acids is measured, and the endogenous flow of all other amino acids is estimated assuming a constant amino acid composition of endogenous protein. Recently, standardized amino acid compositions of endogenous protein have been proposed (Wünsche et al., 1987; Boisen and Moughan, 1996).

In no experiments has the assumption that the total loss of and the amino acid composition of endogenous protein is constant during the growing period of pigs been investigated. It was the objective of the present experiment to test this hypothesis.

Materials and methods

Five growing barrows arising from the matings of Camborough 15 sows to PIC line 326 boars (PIC, Franklin, KY) with an initial BW of approximately 26 kg were prepared with simple T-cannulas inserted into the distal ileum.

The endogenous losses were quantified using a cornstarch-casein diet (Table 1). Casein was the only protein source in the diet, and it was assumed that all dietary proteins were completely hydrolyzed and that all amino acids of dietary origin were absorbed prior to the distal ileum (Kies et al. 1986; Souffrant et al., 1993).

Ileal digesta were collected when pigs reached an average BW of 36, 61 and 95 kg, respectively. During the collection periods, pigs were given ad libitum access to the experimental

diet, but feed intake was recorded on a daily basis. Water was available from nipple drinkers 24 h a day. The initial 5 d of each collection period was considered an adaptation period, and ileal digesta were collected for 12 consecutive hours on d 6 and d 7. Before collections were initiated on d 6, the BW of each pig was recorded.

Feed intake data were summarized at the end of each collection period, and average daily feed DM intake was calculated for each pig and period on a total basis as well as in relation to the metabolic body weight ($kg^{0.75}$) of the pigs.

The endogenous flow of each amino acid was calculated for each pig and period as g lost/kg DMI. The amino acid composition of endogenous protein was calculated by relating the ileal flow of each amino acid to the total endogenous protein flow on a percentage basis.

Results were subjected to repeated measures analysis using the Proc Mixed procedure of SAS (Littell et al., 1996). Treatment means were separated using an LSD test.

Results

Pig weights, calculated metabolic BW, and data for average daily feed intake during each experimental period are given in Table 2. As expected, average daily feed intake increased from period 1 to period 2 ($P < .05$); however, no further increase in feed intake was observed, and average daily feed intake in period 3 was the same as in period 2 ($P > .05$). Because the pigs were heavier in period 3 than in period 2, average feed intake per $kg^{0.75}$ was lower in period 3 ($P < .05$).

Endogenous flow of protein and amino acids per kg DMI are presented in Table 3. The calculated protein loss per kg DMI was higher ($P < .05$) in period 1 as compared to period 2 and period 3, while no difference ($P > .05$) between period 2 and period 3 was detected. Likewise, a higher loss of amino acids was observed in the first period than in the second period with the difference being significant ($P < .05$) for all amino acids except lysine ($P = .054$).

Significant differences ($P < .05$) in the amino acid composition of endogenous protein between period 1 and period 3 were observed for all amino acids except for threonine, histidine, arginine, and cysteine (Table 4). The difference in the composition of endogenous protein between period 1 and period 2 was significant ($P < .05$) only for lysine, leucine, phenylalanine, histidine, arginine, and alanine, whereas significant ($P < .05$) differences between period 2 and period 3 were observed for tryptophan, methionine, isoleucine, phenylalanine, proline, aspartate, serine, and alanine.

Discussion

The effect of body weight on endogenous losses

Pigs had a significantly ($P < .05$) higher endogenous flow of protein and amino acids, expressed as g/kg DMI, at 36 kg than they had at heavier weights (Table 3). Based on this comparison, it would appear that BW *per se* has a significant influence on endogenous protein losses. However, no further decrease in endogenous secretions after 61 kg was observed, and endogenous losses of amino acids and protein were the same in period 2 and period 3. There are two possible explanations for this observation. First, it could be a true physiological mechanism that enables heavier pigs either to secrete less endogenous protein per kg DMI than younger pigs or that enables them to reabsorb larger amounts of endogenous secretions before it reaches the distal ileum because of a more efficient digestive system. Secondly, an alternative explanation for the lack of a decrease in endogenous losses between the 61-kg pigs and the 95-kg pigs could be that feed intake as a function of $kg^{0.75}$ was lower in the heavier pigs. A decreased feed intake would be expected to

increase the amount of endogenous losses per kg DMI (Butts et al., 1993; Stein and Easter, 1997), thus circumventing a possible decrease in endogenous losses due to increased BW of the animals.

Leibholz and Mollah (1988) reported lower losses of endogenous protein per kg DMI in growing pigs fed a protein-free diet as compared to values obtained in younger pigs. They suggested that the relatively higher gut surface area in younger pigs increased the sloughing of epithelial cells into the gut lumen, and thus increased the endogenous losses at the distal ileum. Therefore, endogenous losses will not increase linearly with feed intake as the pigs grow (Leibholz and Mollar, 1988). The results from the present experiment tend to support this hypothesis. However, in a previous study, we compared the endogenous losses in 110-kg growing pigs and adult, gestating sows, and we found no difference between these two groups if the animals were given free access to a protein-free diet (Stein and Easter, 1997). In the present experiment, no difference was found between pigs of 61 kg and 95 kg BW; hence, BW does not seem to influence endogenous losses in animals above 60 kg. In younger animals (less than 60 kg BW), endogenous amino acid losses seem to be elevated per kg DMI as compared to older animals.

The composition of endogenous protein

Significant differences ($P < .05$) in the amino acid composition of endogenous protein were detected among periods, with the differences being particularly large for tryptophan, phenylalanine, the branched chained amino acids, glutamate, and serine. Threonine and cysteine were the only two amino acids with constant ($P > .05$) values relative to total endogenous protein. The results in Table 4 show that not even when the overall protein flow to the distal ileum is constant, as was the case for period 2 and period 3, is the composition of endogenous protein constant. This observation underscores the difficulties in estimating the losses of all amino acids of endogenous origin using the ^{15}N-dilution technique and the homoarginine technique because these two techniques rely on the assumption that the amino acid composition of endogenous protein is constant. Our results suggest that the amino acid composition of endogenous protein in growing pigs is not constant over the weight range from 36 to 95 kg live weight.

Implications

Results of this experiment indicate that values for the total flow of endogenous protein relative to DMI as well as the amino acid composition of endogenous protein may change during the growing period, and that values obtained in younger pigs are not representative for the losses in pigs heavier than 60 kg. Therefore, if values for endogenous losses are used to calculate true or standardized amino acid digestibilities of feed ingredients, care should be taken to ensure that these values are only applied to pigs of the same BW as they were obtained in. Furthermore, calculating true amino acid digestibilities based on a constant composition of endogenous protein may yield unreliable results.

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Table 1. Composition (as is) of the experimental diet[a]

Ingredient	%
Casein[b]	11.7
Soybean oil	4
Cornstarch	70.9
Sugar	4
Solka floc[c]	5
Dicalcium phosphate	3.2
Limestone	.4
Trace mineral salt[d]	.35
Vitamin premix[e]	.2
Chromium Oxide	.25
Total	100

[a] 10 % CP, .7 % Ca, .6% P.
[b] Erie Casein, Erie, IL.
[c] James River, Berlin, NH.
[d] Trace mineral mix provided the following quantities of nutrients per kg of diet: Se, .30 mg; I, .35 mg; Cu, 8 mg; Mn, 20 mg; Fe, 90 mg; Zn, 100 mg; NaCl, 2.87 g.
[e] Vitamin premix provided the following quantities of vitamins per kg of diet: Vitamin A, 5,250 IU; vitamin D_3, 525 IU; vitamin E, 40 IU; menadione K, 2 mg; vitamin B_{12}, .016 mg; riboflavin, 4 mg; D-pantothenic acid, 11 mg; niacin, 15 mg; choline chloride, 110 mg.

Table 2. Weight and average daily feed intake (ADFI) of pigs

Item	Period 1 [a]	Period 2 [a]	Period 3 [a]	SEM [b]
Avg wt, kg	36.5 [c]	61.4 [d]	94.9 [e]	2.6
Avg wt, $kg^{0.75}$	14.8 [c]	21.9 [d]	30.4 [e]	.68
ADFI, kg DM	1.78 [c]	2.39 [d]	2.46 [d]	.08
ADFI, g DM/ $kg^{0.75}$	120 [c]	109 [d]	79 [d]	4.4

[a] Means of five observations.
[b] Pooled standard error of the mean.
[c,d,e] Means within a row lacking a common superscript are different ($P < .05$).

Table 3. Endogenous losses of dry matter, protein, and amino acids (g/kg DMI)[a]

Item	Period 1	Period 2	Period 3	SEM [b]
Dry Matter	129 [c]	114 [d]	119 [cd]	5.4
Protein	21.55 [c]	15.22 [d]	15.06 [d]	1.23
Indispensable amino acids				
Arginine	.52 [c]	.34 [d]	.34 [d]	.04
Histidine	.32 [c]	.19 [d]	.21 [d]	.02
Isoleucine	.80 [c]	.59 [d]	.76 [c]	.06
Leucine	1.14 [c]	.70 [d]	.68 [d]	.08
Lysine	.51 [c]	.46 [cd]	.41 [d]	.02
Methionine	.22 [c]	.14 [d]	.17 [d]	.02
Phenylalanine	.63 [c]	.39 [d]	.31 [e]	.04
Threonine	1.16 [c]	.87 [d]	.78 [d]	.08
Tryptophan	.23 [c]	.16 [d]	.11 [e]	.02
Valine	1.02 [c]	.71 [d]	.76 [d]	.06
Mean	6.54 [c]	4.55 [d]	4.53 [d]	.41
Dispensable amino acids				
Alanine	1.06 [c]	.84 [d]	.65 [e]	.06
Aspartate	1.44 [c]	.94 [d]	1.02 [d]	0.11
Cysteine	.37 [c]	.26 [d]	.26 [d]	.03
Glutamate	2.44 [c]	1.92 [d]	2.28 [cd]	.18
Glycine	1.45 [c]	.94 [d]	.76 [d]	.09
Proline	.97 [c]	.70 [d]	.78 [d]	.06
Serine	1.05 [c]	.75 [d]	1.15 [c]	.09
Tyrosine	.46 [c]	.31 [d]	.27 [d]	.03
Mean	9.24 [c]	6.64 [d]	7.15 [d]	.58
All amino acids	15.78 [c]	11.20 [d]	11.68 [d]	.97

[a] The endogenous loss of protein and each amino acid was calculated as the amino acid or protein concentration in digesta DM multiplied by the relationship between chromium in digesta DM and chromium in feed DM. n = 5.

[b] Pooled standard error of the mean.

[c,d,e] Means within a row lacking a common superscript are different ($P < .05$).

Table 4. Relative amino acid composition of endogenous protein (% of endogenous protein loss) [a]

Item	Period 1	Period 2	Period 3	SEM [b]
Protein	100	100	100	
Indispensable amino acids				
Arginine	2.40 [c]	2.23 [d]	2.26 [cd]	.08
Histidine	1.46 [c]	1.25 [d]	1.41 [cd]	.05
Isoleucine	3.69 [c]	3.87 [c]	5.09 [d]	.12
Leucine	5.22 [c]	4.61 [d]	4.49 [d]	.14
Lysine	2.38 [c]	3.02 [d]	2.75 [d]	.10
Methionine	.99 [c]	.95 [c]	1.14 [d]	.03
Phenylalanine	2.93 [c]	2.56 [d]	2.04 [e]	.09
Threonine	5.35	5.73	5.21	.18
Tryptophan	1.08 [c]	1.02 [c]	.75 [d]	.04
Valine	4.70 [c]	4.64 [cd]	5.06 [d]	.10
Mean	30.21	29.87	30.20	.54
Dispensable amino acids				
Alanine	4.94 [c]	5.51 [d]	4.28 [e]	.12
Aspartate	6.62 [cd]	6.16 [c]	6.78 [d]	.20
Cysteine	1.70	1.73	1.72	.05
Glutamate	11.26 [c]	12.59 [cd]	15.25 [d]	.54
Glycine	6.72 [c]	6.20 [cd]	5.08 [d]	.41
Proline	4.49 [c]	4.63 [c]	5.20 [d]	.16
Serine	4.82 [c]	4.89 [c]	7.61 [d]	.24
Tyrosine	2.14 [c]	2.00 [cd]	1.77 [d]	.08
Mean	42.68 [c]	43.70 [cd]	47.70 [d]	1.09
All amino acids	72.90	73.57	77.90	1.45

[a] Calculated by expressing the concentration of each amino acid in endogenous protein (Table 3) as a percentage of total endogenous protein. n = 5.

[b] Pooled standard error of the mean.

[c, d, e] Means within a row lacking a common superscript are different ($P < .05$).

Development and Evaluation of a Herd Health Monitoring System for Swine Operations[1]

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Introduction

In today's intensive pork production systems, herd health is a major factor determining the profitability of the business. Among the economically most important diseases in the Midwest are porcine reproductive and respiratory syndrome, pseudorabies, transmissible gastroenteritis, swine influenza and pneumonia caused by *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*. Several studies have evaluated the financial impact of acute outbreaks of these diseases in swine operations (Miller & Kliebenstein, 1985, Mullan et a. 1994, Poulson et al., 1993, Rougoor et al., 1996). However, the predominant problem in many swine operations is a subclinical infection of the herd rather than acute outbreaks of diseases. Infected pigs are not obviously sick or die, but perform poorly. The economic consequences of subclinical infection can be slow growth, decreased feed efficiency, low fertility, increased numbers of abortions and stillbirths, and smaller litter sizes (Baysinger et al., 1997). For farms operating on an 'all in, all out' basis, the difference in weight gain between infected and non-infected pigs can be an additional problem, because finishing pigs do not reach market weight at the same time.

The objective of our study was to develop a herd health monitoring system to detect subclinical disease at an early stage. The ability of this system to detect changes in herd health status was evaluated. We also determined the magnitude of the impact of infection with different pathogens on weight gain, carcass quality and reproductive performance. On the basis of this information, the costs and benefits of specific intervention measures (e.g. vaccination, test-and-removal, antibiotic treatment) can be evaluated. This will assist farm managers in decisions on disease management.

Materials and Methods

In 1996, the first year of this two year study, three university farms were monitored. Farm 1 was a pasture operation. On farm 2, the sows and nursery pigs were kept indoors and the grower and finisher pigs outdoors. Farm 3 was a complete confinement operation. Each farm was visited four times, with a 6-8 week interval between visits. On every visit blood samples were obtained from 90 pigs of different age groups. On these samples serological testing was performed to

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detect antibodies to porcine respiratory and reproductive syndrome virus (PRRSV), transmissible gastroenteritis virus (TGEV), swine influenza virus (SIV) and *Actinobacillus pleuropneumoniae* (APP). These infectious agents were chosen for monitoring because of their potential economic impact and at least one of the farms had a recent diagnosis of infection with these pathogens. Each farm had two cohorts of 30 pigs followed from nursery to slaughter. Individual pigs in the cohort were identified with ear tags. At each visit, pigs were weighed to monitor weight gain. Serum samples were collected to monitor changes in antibody titers. Figure 1 illustrates the sampling scheme on each farm for the first project year.

At slaughter, carcasses were examined for gross pathology: the skin was evaluated for mange, the snout for atrophic rhinitis, the lungs for pneumonia and the liver for parasite infestation. In order to evaluate carcass quality, back fat and loin eye area were measured and color, firmness and marbling of the loin eye were scored. To determine the association of subclinical infection with reproductive performance of sows, the farrowing interval, number of liveborn, number of stillborn and weaning weights were recorded. The influence of seropositivity on weight gain in nursery to finisher pigs and on productivity in sows was evaluated using a multiple linear regression analysis.

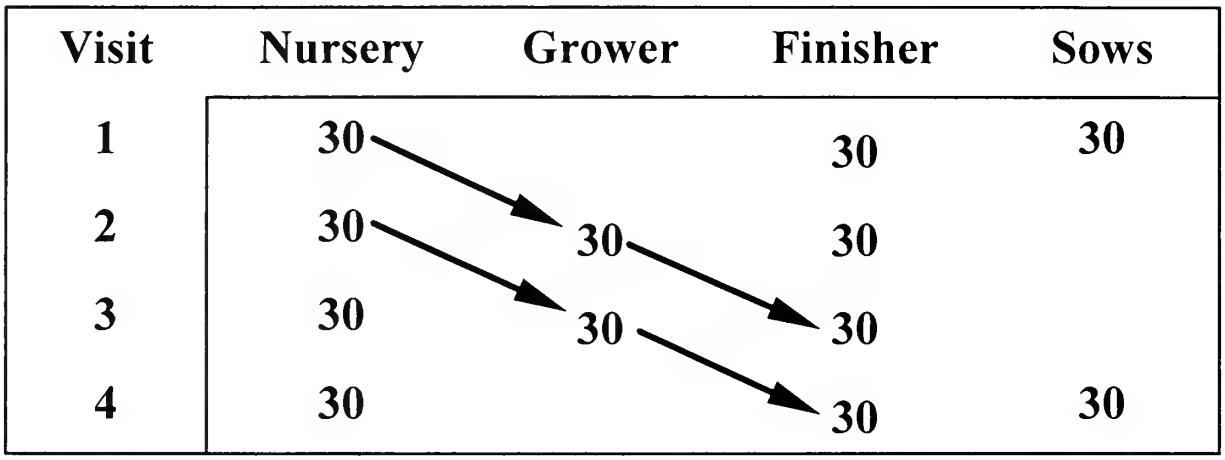

Figure 1. Sampling scheme for farm visits in 1996. Numbers indicate number of pigs sampled from each age class on each visit. Arrows indicate individually identified pigs followed longitudinally.

Results

In all of the three university herds PRRSV was endemic in the sow herd with seroprevalence rates of 11.7%, 6.7% and 51.7% respectively. TGEV infection was prevalent in all herds throughout the year; 18.3% to 56.7% of the sows were seropositive. However, grower and finisher pigs were seropositive for TGEV only sporadically. On all three farms seroprevalence rates were the highest for SIV, with averages of 63.9%, 59.7% and 63.3% respectively. Only one farm had APP seropositive animals during a limited period of time. The surveillance system was effective in detecting changes in disease prevalence ≥15% (α=0.05, power=0.80). Figure 2

shows the prevalence of antibodies against PRRSV, SIV, TGEV and APP on each farm. Figure 3 indicates the prevalence for each age group across farms.

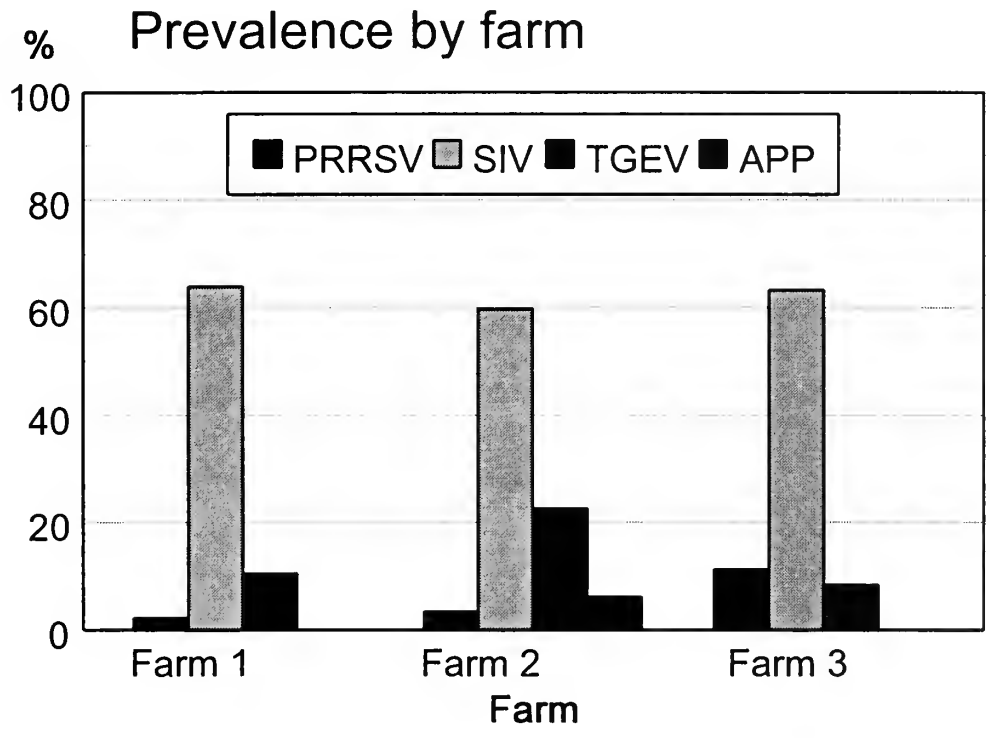

Figure 2: Prevalence of PRRSV, SIV, TGEV and APP on the three university farms

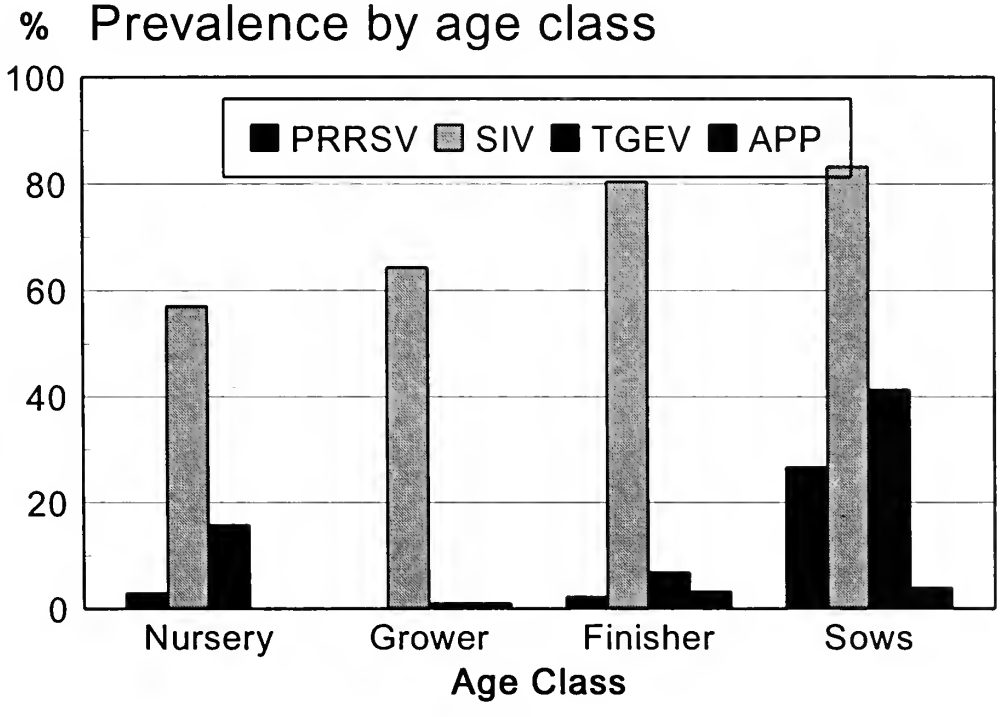

Figure 3: Prevalence of PRRSV, SIV, TGEV and APP in different age groups

A multiple linear regression model was used to evaluate the influence of seropositivity on weight gain. The outcome variable was average daily weight gain (in grams) between the first farm visit (6-8 weeks of age) and the last farm visit (22-24 weeks of age). The mean average daily weight gain (± standard deviation) on farm one was 820 (± 97) grams per day, on farm two 651 (± 65) grams per day and on farm three 738 (± 75) grams per day. For the regression model farm and cohort effects were coded by orthogonal contrast coding. On each farm the two cohorts were compared among each other. The pasture farm was contrasted to the other two farms and the farm that raised finishers outdoors was compared to the complete confinement operation. Other variables included in the original model were age at first bleeding and weighing, sex, positive antibody titers for each of the four diseases at each of the three bleedings and gross pathology at slaughter. A significant influence (at α=0.05) on weight gain was found for farm and cohort effects and age at first bleeding and weighing. Sex was not significant at an α level of 0.05 (p=0.085), but was forced into the model as females have been shown to have a slower weight gain if no split sex feeding is practiced. Nursery pigs with positive titers for SIV at 6-8 weeks of age gained an average of 27 grams per day more than pigs without maternal antibodies at that age. Antibodies for PRRSV in the nursery increased the weight gain by 73 grams per day. The six pigs that seroconverted for PRRSV in the finisher stage had a significantly lower weight gain (-46 grams per day) compared to the negative pigs. Of the findings at slaughter only lung pathology influenced average daily weight gain significantly. For each percent increase in lung tissue with pneumonia, weight gain was decreased by 5 grams per day. Pigs that showed fibrous scars in the lung tissue gained 23 grams per day less than pigs without visible signs of having had pneumonia at an earlier age. In table 1 the variables that remained in the final multiple regression model and their associated β, t, p and sr^2 values are listed.

Table 1: Final multiple linear regression model for average daily weight gain in nursery to finisher pigs. R^2=0.74; F(12,121)=29.13; p<0.0001

	Effect on ADG	**β g/day**	**t**	**p**	**sr^2**
first vs. second cohort farm 1		-27.3	-1.25	0.2121	0.0033
first vs. second cohort farm 2		-9.1	-0.87	0.3849	0.0016
first vs. second cohort farm 3		-45.5	-2.94	0.0040	0.0183
pasture farm vs. indoor farms		227.3	7.43	0.0001	0.1174
indoor vs. partially outdoor farm		81.8	-4.57	0.0001	0.0444
age at first bleeding		-4.5	-2.72	0.0076	0.0157
SIV positive at 6-8 weeks (0=no; 1=yes)	higher	27.3	1.75	0.0411	0.0065
PRRSV positive at 6-8 weeks	higher	72.7	1.89	0.0305	0.0076
PRRSV positive before slaughter	lower	-45.5	-1.69	0.0468	0.0061
% of lung pneumonic at slaughter	lower	-4.5	-2.32	0.0111	0.0114
scars on lung tissue (0=no; 1=yes)	lower	-22.7	-1.68	0.0474	0.0060
sex (0=male; 1=female)	male>female	-13.6	-1.38	0.0851	0.0040

To evaluate the impact of subclinical disease on sow productivity, multiple linear regression models were performed with the farrowing interval, number of liveborn piglets, number of stillborn (including mummified fetuses) and weaning weight as the outcome variables. The weaning weight and the number of liveborn piglets in a litter were not associated with any of the monitored diseases. The mean (± standard deviation) farrowing interval was 149.1 (± 4.7) days on farm one, 146.0 days (± 22.3) on farm two and 135.8 (± 12.7) days on farm three. The farrowing interval was increased by 7.1 days in APP positive sows (p=0.023). Sows vaccinated and seropositive for TGE had a 25.9 days shorter farrowing interval (p=0.006). Vaccinated TGE positive sows also had a smaller number of stillborn or mummified piglets in the litter. The average number of dead pigs in the litter was 2.7 on farm one, 0.9 on farm two and 1.8 on farm three. A positive vaccination titer for TGE decreased this number by 0.6 (p=0.045). The number of stillborn piglets in sows seropositive for SIV was increased by 0.9 (p=0.018). None of the sows were vaccinated for SIV. Seropositivity for PRRSV did not influence any of these measures of reproductive performance.

Discussion

The herd health monitoring system was effective in detecting changes in herd health status on the three farms monitored during the first year of the study. In the multiple linear regression model for average daily weight gain of nursery to finisher pigs antibody titers were responsible for only a small part of the variation in weight gain. Most of the variation in weight gain could be attributed to farm and cohort effects. Of the total variation in weight gain, 1.41% could be explained by antibody titers at 6-8 weeks and 0.61% by seroconversion for PRRSV in the finisher phase. This could mean that subclinical disease has only a small impact on performance compared to feeding, genetics and management factors. However, the farms chosen for this study represented a broad range of management types from a pasture operation to complete confinement. Therefore the high variation between farms is expected. Antibodies for SIV or PRRSV at 6-8 weeks of age had a positive effect on average daily weight gain. This indicates that the titers measured are from maternal antibodies rather than from infection. Most of these seropositive pigs were seronegative at the second bleeding at 16 weeks of age. There was also a positive correlation between antibody titer and weight in 6-8 week old nursery pigs.

Of the subclinical diseases monitored, only antibodies for PRRSV had a significant impact on average daily weight gain. However, in the groups monitored, the prevalence of antibodies for TGEV and APP were too low to detect an impact of these diseases on weight gain. More than 80% of the finisher pigs seroconverted for SIV. Thus a moderate decrease in weight gain due to this infection may have remained undetected because the sample size of seronegative pigs was too small. The second year of this study includes a greater number of farms as well as a slightly increased cohort size. Therefore the evaluation of the magnitude of the impact of TGEV and SIV on weight gain will be facilitated. The prevalence of APP was low on all farms, it might not be an important pathogen for subclinical infection in the area we were doing our research in.

Fertility in sows was significantly decreased by several of the monitored diseases. While litter size and weaning weights were not affected, the number of stillborn and mummified piglets as well as the length of the farrowing interval were influenced by the health status of the sows. Positive titers in unvaccinated sows increased the number of stillborn piglets and increased the farrowing interval. Positive titers from vaccination, on the other hand, improved reproductive performance. It was surprising that positive titers for PRRSV did not have an effect on fertility in the sows monitored in this study. Sows can remain seropositive for PRRSV for a long time after infection. The data that we evaluated included only the last farrowing before our visit to the farm. Therefore an earlier infection with persistent antibodies might not have an impact on reproductive performance during our measuring interval. However, Baysinger et al. (1997) observed a decrease in reproductive performance that lasted for one year after infection with PRRSV. For the continuation of the project in 1997, the sows are identified individually and blood samples are collected twice in a 6 month interval. Thus, we will be able to evaluate possible changes in serological status over time.

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Polymerase Chain Reaction (PCR) -Based Assay for Detection of *Eperythrozoon suis* Infection in Pigs

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Introduction

Eperythrozoon suis is a red blood cell parasite.[1] The disease, eperythrozoonosis, is characterized by four syndromes: (I) decreased reproductive efficiency of sows, (ii) weakness and anemia in baby pigs and increased incidence of enteric and respiratory infections, (iii) delayed production gains in feeder pigs, and (iv) acute hemolytic anemia in feeder pigs.[2] Although this disease was first reported in the United States in 1932, our understanding of this organism and the disease it causes remains largely incomplete.[3]

Diagnosis of acute infection with *E. suis* is usually based on direct microscopic observation of organisms attached to red blood cells. However, this method has limitations because the presence of organisms in an acutely infected pig is transient and in chronically infected pigs very few organisms are found.[1] Serologic tests for the diagnosis of eperythrozoonosis have also been reported. These tests have diagnostic limitations due to a marked variability in antibody response, as well as a failure to identify acutely infected pigs.[4,5] The lack of an efficient test for diagnosing acute and chronic *E. suis* infection has resulted in tremendous controversy concerning the true impact of this disease in pig populations.

The objective of our research studies was to develop a PCR-based test for diagnosing eperythrozoonosis. Future studies will attempt to answer questions related to the prevalence of *E. suis* in pig populations and establish the usefulness of this assay for detection of *E. suis* in pig tissues to be used for human transplantation.

Experimental Design, Results, and Application:

Our hypothesis is that both sick pigs and pigs with inapparent infections caused by *E. suis* can be detected using PCR amplification of a gene specific for this organism in a blood sample. Our assay targets a ribosomal gene of this organism.

To test this hypothesis we designed a set of primers based on sequence data of the ribosomal gene that were specific for *E. suis*. This figure shows an example of our **PCR results using a primer set that was not specific for *E. suis***. Lane 1, is a DNA size marker; Lane 2, E. suis; Lane 3, Haemobartonella felis; Lane 4, Bartonella bacilliformis, and Lane 4, Mycoplasma genitalium.

This figure shows an example of our **PCR results using the primer set we designed that was specific for E. suis.** Lane 1, is a DNA size marker; Lane 2, E. suis; Lane 3, Haemobartonella felis; Lane 4, Bartonella bacilliformis, and Lane 4, Mycoplasma genitalium. Notice that PCR product is found only in lane 2, therefore the test is specific for *E. suis*. Our preliminary work has shown that this primer set did not bind to DNA from various organisms genetically related to E. suis.[6] The limits of detection of our PCR assay were comparable to what has been previously reported. It was determined that between 18 to 1138 organisms were required to obtain a positive test result.[7]

This PCR-based assay is rapid, sensitive and specific for the detection of *E. suis* infection. In addition, the assay is easy to perform and lends itself to routine analysis. The diagnostic potential of this assay needs to be further evaluated in a farm setting. We will attempt to answer questions related to the prevalence of *E. suis* in pig populations, the risk factors associated with positive PCR assay results, and transmission and control of this disease. By testing seedstock, we can identify infected pigs before they are introduced into a clean herd. This PCR-based assay will serve not only as a tool to help us better understand the role this organism plays in diseases of pigs, but will be of tremendous value in testing pig tissues for this organism before they are used for human transplantation.

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The six pages which follow summarize a Center proposal for a National Consortium for Pork Technology and Education. I, along with co-PIs from five other academic institutions, were successful in being one of 35 proposals, from among 461 submitted, selected to develop full Center proposals to be submitted to the Fund for Rural America (FRA). Iowa State University, under the direction of Dr. Jim McKean, has been designated the coordinational institution, recognizing simulateously the extensive role of all six institutions. If we are successful at the next level (it is anticipated that 17 or 18 proposals will be chosen from among the 35), we will receive FRA full program awards from USDA to develop the Center.

Part of a guiding principle to be used in our Center is to have an on-going dialogue between stakeholder groups with an interest in swine production in the state of Illinois. To that end, I have submitted the following six pages which summarize our FRA proposal that was developed and submitted in March 1997. I invite anyone reading this proposal who would like to have input into the thought process of the Consortium as we develop the final proposal (to be submitted in March 1998) to call or write me: Gay Miller, 2001 S. Lincoln Ave, Department of Veterinary Pathobiology, Room 2522 VMBSB, Urbana, IL 61802; phone 217-333-2449; e-mail: gymiller@uiuc.edu. We are very interested in your feedback to our proposal, and to your contributions suggesting the most important issues the pork industry is facing in the state of Illinois.

We envision that there will be a large group of people involved in the Center, both directly and indirectly. We know that current constraints to the pork industry are complex. There are a wide range of needs to address. The input of many people with diverse backgrounds and expertise will be required to adequately address the needs. I look forward to hearing from you.

National Consortium for Pork Technology and Education

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PROJECT SUMMARY

The problems and issues confronting today's swine industry are complex, cross political and geographic boundaries and exceed the resources of any single university, agency, or organization to effectively resolve. Therefore, a national "network of excellence" is needed to effectively reduce obstacles that are constraining the US swine industry from capitalizing on the growing world demand for pork. The National Consortium for Pork Technology and Education (NCPTE) is a collaborative effort of veterinary colleges at six land-grant universities. Members of the Consortium have a common interest in combining their efforts in research, extension and academic programs to strengthen development of sustainable technology and to transfer this technology to the swine industry. The Consortium will work collectively with producer groups, community leaders, state and federal agencies, and other stakeholders to coordinate development and implementation of programs to address the critical issues facing the pork industry and consumers.

The Consortium goals are, in general:

- Assess emerging pork production technologies for production efficiency, for sustainability, and for consumer and environmental acceptance.
- Address community concerns about growth of and technological changes in the pork industry.
- Implement on-farm food safety and product quality enhancement programs using Hazard Analysis Critical Control Point (HACCP) methodology and other technologies.
- Implement cost effective world-class pork production and marketing systems.

PROJECT NARRATIVE

Center Concept

In 1996, for the first time in over 40 years, US pork exports exceeded imports. These exports generated over $270 million to the producers in increased market prices. World demand for pork is expected to increase at an accelerated rate because of globalization of agricultural markets. This increased demand for pork provides a rare opportunity for long-term economic strengthening of America's pork producers and their rural communities. While the US is strategically blessed with the natural resources to meet this increased domestic and global demand for pork, ***several urgent technological, environmental and social problems threaten the US swine industry's opportunity to capitalize on this increased demand for pork***. These problems and issues are complex, transcend political and geographic boundaries and cannot be effectively resolved by any single university, agency, state, or organization. Instead an entity with national scope and impact is needed to identify and find solutions to the problems that are constraining development of the swine industry in the US.

The National Consortium for Pork Technology and Education (NCPTE) is proposed as a collaboration of veterinary and agriculture colleges at six land-grant universities (Iowa State University, North Carolina State University, Purdue University, University of Illinois, University of Minnesota and University of Wisconsin. Members of the NCPTE have a common interest in combining their efforts in research, extension and academic programs to strengthen development of sustainable technology and to transfer this technology to the US swine industry. The NCPTE members will work collectively with producer groups, community leaders, state and federal agencies and other stakeholders to coordinate development and implementation of programs to address the critical issues facing the pork industry of today and tomorrow.

Mission Statement

The Consortium is an interdisciplinary, multi-institutional catalyst for development of science-based technologies and educational programs that support competitive pork production systems in the US. The NCPTE will encourage sustainable practices that facilitate competitive world-class pork production and meet public needs and perceptions for environmentally friendly, community-based production systems.

In order to accomplish its mission, the NCPTE will focus its efforts on four strategic problem-oriented goals. The attainment of these goals will strengthen the pork industry, the rural communities in which it exists, and will enhance the competitiveness of the industry in the global marketplace.

Overall Goals

The overall goals of the Consortium will be:

- Development and implementation of systems for rapidly assessing applicability of emerging technologies and for guiding pork producers in the prompt adoption of technologies that are sustainable and environmentally acceptable.

- Development and implementation of procedures for addressing community concerns about growth and technological changes in the pork industry so that socially and environmentally acceptable expansion of the industry can occur in areas where the strategic resources are abundant.

- Development and implementation of programs for continuously improving pork quality and the safety of pork products through rapid introduction of new technologies and of strategic on-farm procedures based on the Hazard Analysis Critical Control Point (HACCP) methodology.

- Development and implementation of pork production and marketing systems that provide domestic and global consumers with products that they demand at the most competitive prices in the world market.

Bridging Research, Education and Extension

The Consortium was formed to respond to an environment in which reductions in public and private funds have forced individual institutions to more narrowly focus their educational and research efforts. Yet it is at this very time that pork producers and their rural communities are requiring new and comprehensive answers to an expanding and complicated array of ***critical national issues.*** The NCPTE is designed to efficiently facilitate and coordinate the multidisciplinary and interinstitutional networks of excellence which are needed by the US pork industry to help them to respond to this new domestic and international environment.

As land-grant universities, members of the Consortium enthusiastically embrace the integration of research, teaching and educational programs, and have a tradition of working closely with producers, state and federal agencies, processors and consumers. The NCPTE will fulfill those functions by providing science-based information to such stakeholders through development and implementation of integrated programs in four focus areas: 1) Community Issues; 2) Production Technology Issues; 3) Social and Policy Issues; and 4) Consumer Issues.

Each participating university contributes unique resources and areas of expertise to the Consortium's efforts. The NCPTE offers a diverse, and complementary aggregation of social and technologic academic talents, research and educational resources, geographic and social insights, and a unique access to and understanding of farm-to-fork pork production systems, societal concerns, and community development issues representative of the entire US pork industry. The NCPTE will develop technologies and implement solutions to support a sustainable pork industry and the rural communities in which that industry exists. Substantial efficiencies will be gained in developing and implementing research, education and extension efforts that will meet the needs of the entire US swine industry because of the participation in this Consortium by universities from major swine-producing states.

Planning Activity

This Consortium was formed because of a common interest in nurturing collaborative research, extension and academic networks to strengthen development and transfer of socially- and environmentally-acceptable technology to the swine industry. Various faculty and administrative leaders from the six universities have already met on several occasions to form issue teams and to develop strategic goals for the Consortium.

A Fund for Rural America (FRA) Center Planning Grant will support prioritization and planning activities conducted with critical stakeholders such as producers and producer organizations, societal and community leaders, and governmental agencies. These planning processes will include several activities:

Listening and Planning Forums
The NCPTE will sponsor geographically-based planning forums with industry leaders, representatives of state and federal agencies, societal and community leaders and others to determine appropriate programs and approaches to achieve the stated goals within the four strategic focus areas. Representatives from each Consortium university will attend the forums to expose individuals from varied geographic areas and production expertise to problems and issues of the other areas. These forums will facilitate the identification of partners who will provide financial and technical assistance, and who will become collaborators in preparing the FRA proposal and in fulfilling the goals of NCPTE. For example, we expect to develop collaborations with pork producer organizations in each state, the National Pork Board, the National Pork Producers Council, state community-development agencies, state and federal environmental protection groups, state and federal agencies, the pork processing industry, and export groups.

Information Searches
Subcommittees from the Consortium will identify successful interdisciplinary centers to ascertain the strengths, weaknesses and benefits of their respective organization and operational styles. These subcommittees will also develop an inventory of state strategic plans for development of agricultural enterprises and of complementary activities of member institutions and groups and organizations within their states, including the proposed activities of other FRA Centers.

Stakeholder Input into Center Proposal
The stakeholder collaborators identified in the Planning Forums will assist in the preparation of the FRA Center proposal. These collaborators will provide feedback from stakeholder groups during the proposal development process and critical review of proposal drafts during all stages of the preparation process. After formal establishment of the NCPTE, these collaborators will form the nucleus of advisory and assessment boards designed to ensure that the NCPTE is successful in fulfilling its mission.

Final Proposal Preparation
From stakeholder inputs, draft documents and information searches, writing subcommittees will prepare a draft of programmatic objectives, research and educational priorities and budget to attain each goal. Drafts will be critically reviewed at multiple stages by other writing team members,

stakeholder collaborators from the private and public sectors, and Consortium institutions, and integrated into a comprehensive final proposal and budget.

Collaborative Arrangements

The Consortium represents collaborations among land-grant universities in Iowa, Illinois, Indiana, Minnesota, North Carolina and Wisconsin. Within each of these universities, faculty from Colleges of Veterinary medicine, Colleges of Agriculture, and other divisions, such as social sciences, environmental sciences, economics, and political science will become members of interdisciplinary issue teams to address each goal. The National Pork Producers Council and its affiliates, other producer groups, Departments of Agriculture, State and Federal agencies, and rural development groups in each state will be partners in the activities of the NCPTE. The NCPTE will develop links with other centers such as the proposed National Center for Manure and Animal Waste Management, and will form similar close working relationships with existing centers and institutions, such as the North Central Regional Center for Rural Development.

The Consortium will work with the existing Animal Production Food Safety Consortium and the Food Safety Consortium in basic and applied food safety research efforts, and the National Pork Producers Council, APHIS, FSIS, and private accredited veterinarians in developing and implementing programmatic on-farm HACCP certification activities and in the development of products to meet consumer demands worldwide. Through these interactions, on-farm certification systems based upon HACCP principles can be implemented that enable low resource and individual pork producers to access the world markets, and to maintain the continuous product improvement that will increase US pork acceptance worldwide.

A strength of this collaboration derives from the access to diverse production systems by the Consortium institutions--from integrated pork production/processing systems to concentrations of diversified livestock and crop operations. This diversity of resources, production expertise and challenges lends itself well to cross-fertilization of ideas and skills that will generate innovative solutions to strengthen the entire US pork industry. Proposed production modifications and emerging technologies can be demonstrated in a variety of systems under uniform comparative measurements to insure that the results are as expected in diverse regions and production systems. This collaboration will be particularly useful in development and testing of control points for microbes of food safety interest. Such activities may speed the characterization of the most appropriate food safety-related on-farm control points, thereby benefitting producers, processors and domestic and international consumers. Diversified and low resource producers will benefit substantially from this approach because they will not be individually required to develop, test and implement new control points, but merely adopt those most applicable to their operations.

Effects of Fumonisins on Immune Function in Swine

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Introduction

Fumonisins, mycotoxins found in corn and corn-based feed worldwide, can induce liver damage and, at high concentrations, fatal pulmonary edema in swine (Haschek *et al.*, 1992; Motelin *et al.*, 1994; review by Dutton, 1996). However, reports on the immune effects of fumonisins in swine are conflicting. Osweiler *et al.* (1993) found that pigs fed a diet containing 33 mg fumonisin/kg for 21 days had a decreased titer and lymphocyte blastogenic response to pseudorabies vaccination at 7 but not 14 days. Rotter *et al.* (1996) reported a dose-dependent decrease in the blastogenic response of lymphocytes isolated from pigs fed diets containing 0, 0.1, 1, or 10 mg fumonisin/kg diet for 8 weeks. However, lymphocytes isolated from sows fed diets containing 100 ppm fumonisin for 17 days did not respond differently from controls in the blastogenesis assay (Becker *et al.*, 1995). We recently demonstrated that fumonisin ingestion by pigs decreases the clearance of particulate material and bacteria from the blood (Smith *et al.*, 1996).

Fumonisins inhibit an enzyme in the biosynthetic pathway of sphingolipids causing dramatic alterations in the concentrations of sphinganine and sphingosine (Wang *et al.*, 1991; Riley *et al.*, 1994). Because sphingoid bases and complex sphingolipids have varied and complex roles in cell signaling pathways and are important components of the cell membrane, inhibition of sphingolipid biosynthesis has been implicated as a mechanism of fumonisin-induced toxicoses (Hannun and Bell, 1989; Merrill *et al.*, 1996). Sphingosine inhibits protein kinase C which activates downstream signaling events that result in the production of the cytokine, tumor necrosis factor (TNF). This cytokine is produced by macrophages and helps to stimulate the immune response.

Therefore, we hypothesized that exposure of swine to fumonisin impairs both specific (B and T lymphocyte) and non specific (T lymphocyte, macrophage) immune function, and that inhibition of sphingolipid biosynthesis is the mechanism of this impairment.

Objectives

1. To examine the effects of fumonisins on phagocyte functions.
2. To determine if fumonisins affect the ability of phagocytes to orchestrate the immune response.

3. To examine the effects of fumonisins on specific immunity.
4. To determine the effect of fumonisins on sphingoid base concentration in immune cells and organs.

Methods and Results

Objective 1. To examine the effects of fumonisins on phagocyte functions.

Pulmonary alveolar macrophages (PAMs) were obtained either from untreated pigs or pigs fed fumonisin-containing culture material at a dose of 15 mg fumonisin B_1/kg for 4-5 days or up to 7 mg/kg for 5 weeks. To examine bacterial attachment and killing, PAMs were incubated with opsonized Salmonella at 37°C for up to 2 hours.

Fumonisin exposure consistently inhibited the phagocytic activity of alveolar macrophages after *in vivo* exposure. Fumonisin inhibited phagocytic activity and bacterial adherence of PAMs, however, the rate of killing did not seem to be affected. The production of superoxide was not affected either. Inhibition of phagocytosis may increase susceptibility to infectious diseases and extend recovery from other diseases.

Objective 2. To determine if fumonisins affect the ability of phagocytes to orchestrate the immune response.

Tumor necrosis factor (TNF) production and activity was determined in PAMs obtained either from untreated pigs or pigs fed fumonisin-containing culture material at a dose of 15 mg fumonisin B_1/kg for 4-5 days. PAMs were incubated with and without LPS and PMA for 12-15, 24, and 36 h and TNF production was determined by the standard WEHI killing bioassay (Wollenberg *et al.*, 1993).

PAMs from treated pigs had decreased production of TNF as compared to similarly stimulated PAMs from control pigs. These data indicate that dietary exposure to fumonisin may inhibit the ability of swine to mount an immune response.

Objective 3. To examine the effects of fumonisins on specific immunity.

The response to vaccination was compared between control and fumonisin fed pigs to examine the effects of fumonisin on humoral and/or cell mediated immune responses to specific antigens. Pigs were fed fumonisin-containing culture material at a dose of up to 7 mg/kg for 5 weeks and were vaccinated with a killed pseudorabies virus (PrV) vaccine on weeks 1 and 3. Humoral response was determined by measuring the antibody titer to the vaccine antigen by serum neutralization (SN) at 0, 3, and 5 weeks. The cell mediated response was determined by the blastogenic response to vaccine antigens. [^{3}H]Thymidine uptake after incubation with media, PHA (+ control), and PrV was measured. Changes in cell mediated immunity was further examined by quantifying the number of CD3, CD4, and CD8 positive T lymphocytes in the

blood by flow cytometry to determine the numbers of T cells, T helper cells, and cytotoxic T cells.

There were no differences in SN titers to PrV at any time points. A PrV index showing a blastogenic response of PrV over media increased in treated pigs on Week 5. There were no major differences in the relative numbers of the different lymphocytes except at week 5 when there was a slight increase in the numbers of CD3 positive, CD8 positive, and double positive T cells in the treated pigs. These results indicate a potential slightly beneficial effect of fumonisin in enhancing the response to vaccination.

Objective 4. To determine the effect of fumonisins on sphingoid base concentration in immune cells and organs.

Neutrophils, monocytes, and alveolar macrophages were obtained either from untreated pigs or pigs fed fumonisin-containing culture material at a dose of 15 mg fumonisin B1/kg for 4-5 days. The concentrations of sphinganine and sphingosine were determined in these cells as well as the thymus and mesenteric lymph nodes by extraction and HPLC analysis as described by Riley *et al.* (1994).

Elevations in sphinganine and sphingosine were present in neutrophils, monocytes, and alveolar macrophages from fumonisin-treated pigs. Only monocytes and alveolar macrophages had an increased sphinganine to sphingosine ratio. The thymus and mesenteric lymph node had elevated sphinganine and sphingosine concentrations, and sphinganine to sphingosine ratio. Therefore fumonisin exposure increased the concentrations of sphingoid bases in both immune cells and organs.

Discussion

The effects of fumonisin exposure on specific and non specific aspects of the immune response were examined. Phagocytic activity was decreased in PAMs exposed to fumonisin *in vivo*. The bacterial killing assay was used because it more completely tests macrophage function, not only adhesion but also uptake and killing of bacteria. Fumonisin did decrease the ability of PAMs to attach and phagocytize opsonized *Salmonella* sp. in both the high and low dose *in vivo* studies. The rate of bacterial killing and superoxide production was not altered. This suggests a possible problem with Fc receptor synthesis or expression. Therefore some aspects of the pig's immune defenses in organs as lungs, liver, gut, spleen, may be compromised with fumonisin exposure.

To mimic a field situation, pigs were exposed to a low dose of fumonisin and PrV vaccination was used to analyze the specific immune responses to this antigen. The blastogenesis studies showed that long term low dose fumonisin ingestion may slightly enhance the pig's specific immunity in its ability to respond to vaccination. Similarly, flow cytometry showed slightly increased numbers of T lymphocytes, T helper cells, and double positive T cells, indicating that

fumonisin treatment may better prepare the pig to mount a cellular immune response. Fumonisin consistently altered levels of sphinganine and sphingosine in neutrophils, monocytes, and alveolar macrophages, as well as in the thymus and lymph nodes. The significance of these alterations in affecting immune function is unclear but changes in the phagocytes may be limited to perturbations at the receptor level on plasma membranes. Any changes in the immune system may affect susceptibility to disease as well as the response to vaccination. These studies need to be repeated with particular emphasis on the ability of PAMs and neutrophils to adhere to and phagocytize bacteria.

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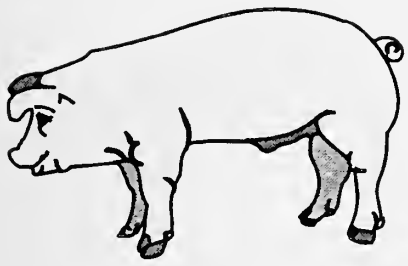

1999 UNIVERSITY OF ILLINOIS SWINE RESEARCH REPORTS

Technology for the 1990's and Beyond

College of Agricultural, Consumer and Environmental Sciences

UNIVERSITY OF ILLINOIS
EXTENSION
College of Agricultural, Consumer and Environmental Sciences

Metric Conversions

Some of the results in this proceedings are reported in the English system (pounds, inches, etc.). However, all scientific meetings and journals require that data be presented in the metric system (kilograms, meters, etc.). Therefore, some of the papers in this proceedings may be reported in the metric system. Carefully observe the units to properly understand each paper. Following are some of the conversion factors that may be useful to you in understanding metric measurements.

Metric		**English**
Length		
1 millimeter (mm)	=	0.03937 inch
1 centimeter (cm)	=	0.3937 inch
1 meter	=	39.37 inches
	=	3.281 feet
	=	1.094 yards
1 kilometer (km)	=	0.6214 mile
Area		
1 square centimeter (cm^2)	=	0.155 square inch
1 square meter (m^2)	=	1.196 square yards
	=	10.764 square feet
1 hectare (10,000 m^2)	=	2.471 acres
1 square kilometer (km^2)	=	0.386 square miles
	=	247.1 acres
Weight		
1 gram (g)	=	0.03527 ounce
1 kilogram (kg)	=	35.274 ounces
	=	2.205 pounds
1 metric ton (t)	=	0.984 ton (long)
	=	1.102 tons (short)
	=	2204.6 pounds
Volume per unit area		
1liter/hectare (l/ha)	=	0.107 gallons/acre (U.S.)
Temperature Conversions		
Centigrade (Celsius)	=	5/9(Fahrenheit - 32)
Fahrenheit	=	9/5(Celsius + 32)

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CONSIDERATIONS IN CONSTRUCTING DIETS FOR NURSERY PIGS

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Overview

The main targets of a nursery pig nutritional program are to (1) avoid postweaning growth depression; (2) smoothly adjust pigs to a low cost, corn-soybean meal-based diet; and (3) accomplish this transition as soon as possible following weaning. These goals can be achieved by a three-phase feeding program that introduces low-cost ingredients in a progressive way, to gently enhance maturation of the gastrointestinal system, and at the same time to supply special ingredients that will encourage maximal growth performance. Such a feeding program when combined with high-health status, high-lean genetics, and high-management standards enables weaning to take place at an age of 3 weeks or less.

Usually, nursery diets are offered for *ad libitum* consumption for a period of at least 4 to 6 weeks following weaning. Initially, a highly complex and quite expensive diet is fed for 4 to 10 days postweaning. Afterwards, a less complex and less expensive diet is offered for 1 to 2 weeks, followed finally by a simple fortified corn-soybean meal-based diet with minimal quantities of milk products. A typical phase feeding program for pigs weaned at 3 weeks of age is presented in Table 1.

Ingredient Selection

Corn is the main energy source for most nursery diets, but sorghum grain and wheat can sometimes be used, especially in areas where production of such cereals surpasses corn production. Barley (regular or hulless), oats (naked or rolled), and triticale are less desirable ingredients, especially for phase I diets. Oat groats are no longer considered a premium ingredient because price does not justify their inclusion in complex diets. Furthermore, thermally processed (i.e., cooked) cereals should be avoided because recent university trials have failed repeatedly to demonstrate any positive effects.

The major protein source is soybean meal, preferably dehulled (46 to 48%). Although soybean proteins are believed to cause allergic reactions in weaned pigs, it has been proved that exposing the young pig's immature gastrointestinal system to increasing concentrations of soybean meal results in better overall performance compared to feeding non-allergic purified soybean proteins and postponing the encounter with soybean meal for a later age. Therefore, postweaning diets (Phase I and II) usually contain a fair amount of soybean meal (15 to 30%). Not only is there no performance loss when soybean meal is included in complex diets, but surprisingly, feeding diets devoid of soybean meal during the first week postweaning has often resulted in subsequent reduced growth performance. Other plant protein that can be used are canola meal, peas, high-protein cottonseed meal, and peanut meal.

Nursery diets should contain milk products. Dried whey, skim milk, and whey protein concentrate are the most commonly used products but whey is currently the ingredient of choice in terms of cost and animal performance. Skim milk is widely considered as the best source of milk proteins and sugars, but recent experiments have indicated that subsequent feed intake may be depressed in pigs fed skim milk when compared to whey-fed pigs. On another front, crystalline lactose has been shown to effectively replace all or part of the whey-associated lactose and thus, its use has become increasingly common. Other sources of simple sugars that can replace milk sugars are whey permeate, sucrose, dextrose, and molasses. Lactose concentration should be kept to a minimum of 25% in Phase I diets. However, it can greatly vary in the diets for the other two phases (from 0 to 20%) depending on health and performance.

The ingredient that revolutionized nursery pig feeding is spray-dried animal plasma protein, introduced in the late eighties. Despite being expensive, plasma protein is considered absolutely essential because it increases feed intake substantially. Best results are obtained when plasma protein is offered during the first few weeks postweaning at concentrations of 2 to 8%. However, beneficial effects are less pronounced in high-health units as well as in late nursery pigs. An array of protein sources has been used in an attempt to mimic the effects of plasma protein at a lower cost. Menhaden select fishmeal, spray-dried red blood cells, spray-dried wheat gluten, and spray-dried egg protein have yielded variable results, and thus none is currently recommended as replacement for plasma protein. Porcine and bovine plasma proteins are equally efficacious in promoting growth performance.

Newly weaned pigs do not utilize lipids as effectively as late-nursery pigs. However, fat concentrations are relatively high in Phase I and II diets (up to 8%) to enhance pellet quality and reduce air-borne dust. The exact concentrations should reflect the concentration of dietary milk products and the expertise of the pellet mill operator. A variety of fats, oils and industrial mixtures can be used, but high quality lipid sources such as soybean oil, corn oil, lard, and choice white grease are strongly suggested for nursery diets.

Salt is an old ingredient that has received considerable attention lately. Young pigs appear to benefit from salt concentrations higher than those necessary to meet the sodium and chlorine requirements. Although the mechanism of action is still unknown, current recommendations call for are at least 0.5 and 0.4% added salt in Phase I and II diets, respectively.

Major growth promotants for young pigs are feed-grade antibiotics, zinc oxide, and copper sulfate. Other additives such as organic acids, chromium, probiotics, yeasts, enzymes, sweeteners, herbs are not well established in terms of their functions and practicality and thus their use is hard to justify. Of course, nursery diets should always be fortified with vitamins, trace minerals, calcium, and phosphorus (via mono or dicalcium phosphate). Phytase (700 to 1,000 units/kg) can be used to lower phosphorus excretion and replace up to 0.10% supplemental inorganic phosphorus.

Nutrient Specifications

Because of their special nature, complex diets are relatively high in energy, with values ranging from 3,500 to 3,700 kcal of DE/kg, depending on fat concentrations. Protein concentrations are also exceptionally high (20 to 25%) in the first two phases, because of the numerous high-protein ingredients routinely used. There is, however, a current trend to reduce protein levels by using more crystalline amino acids, as they become available at more competitive prices. Phase I and II diets are usually overfortified with lysine (as much as 30% above requirements) because young pigs appear to respond to higher doses. However, it is strongly recommended that the concentration of remaining amino acids be calculated using the ideal amino acid profile concept on the basis of the actual lysine requirements (Table 2).

Calcium and phosphorus requirements should be kept to a balance of 1.2-1.5:1.0 (2-3:1 ratio of calcium to bioavailable phosphorus). Up to 3,000 and 2,000 mg/kg of zinc from zinc oxide should be added to Phase I and II diets, respectively, to boost growth performance and reduce diarrhea. In Phase III diets, copper sulfate is generally used instead of zinc oxide. Growth-promoting benefits of zinc oxide and copper sulfate are not additive and thus they should not be used together. However, either of these compounds provides additive benefits with antibiotics.

Recent evidence suggests that nursery pigs might benefit from B-vitamin concentrations higher than those currently recommended. However, data are not clear regarding which B vitamin may be eliciting the response. Certainly, today's high-lean genotypes may, indeed, require higher levels of certain B-vitamins than those suggested by the 1998 NRC Committee. More research is clearly needed on this subject.

Feed Manufacturing

Suggested particle size for grinding corn is 600 μm or less. Complete-diet particle size should range from 600 to 800 μm. Phase I and II diets preferably should be pelleted but Phase III diets can be fed in a meal form. Although young pigs do not appear to benefit from a small pellet size, Phase I and II diets are usually pelleted to a size of 2 to 4 mm. In general, all nursery diets can be pelleted with a single die of 4 to 6 mm in diameter with no problems in growth performance.

Modern feed technologies such as extrusion and expansion that are widely used in Europe have not succeeded in replacing pelleting in North America, primarily because many university trials have failed to demonstrate any advantages in terms of growth performance. Thus, cooked cereals are not advantageous in modern complex diets.

Feed Budgeting

The allotment of feed in the different phases and the timing of diet changes can be based on three different approaches. Diets can be offered for ad libitum consumption for a specific period of time (e.g., 1, 2, and 2 weeks for each of the Phase I, II, and III diets, respectively) but overfeeding of the most expensive diets should be expected. Another approach requires that diet changes be made at specific body weights. This is based on the fact that diet specifications have been set with specific weight ranges as targets. However, this method requires frequent weighing of large groups of pigs, because performance is not easily predicted in the nursery.

A more user-friendly method that avoids both overfeeding of the expensive diets and undernourishment in critical stages of growth is feed budgeting. In this feed management system (Table 3) a predetermined (based on weaning weight) amount of each diet is allotted before the next diet is introduced (e.g., 2, 8, and 16 kg per pig of Phase I, II, and III diets, respectively).

This system needs to be farm-specific to be successful because the exact amount of each diet varies with weaning weight and subsequent growth performance. Weaning weights have a standard deviation of around 1 kg (Table 4). With this information and the average weaning weight of each farm, a feed budget worksheet can be easily drawn (Table 5). For example, in a 1,000-head nursery with an average weaning weight of 6 kg, four weight groups can be created. First the number of pigs (column A) falling in each weight category (column A) are calculated based on the percentages found in Table 4. Then, four (or more) weight groups are formed based on farm preference and ability to handle multiple groups (column C). The number of pigs in each weigh group is in column D. From Table 3, the total feed allowance per pig (column E) is multiplied with the number of pigs in each group (column D) to calculate total feed per group in kg (column F) and number of bags (25 kg each; column G). Also, the number of pens required for each group (column H) can be calculated by dividing the number of pigs in each group by the desired group density (set here at 20 pigs/pen). Finally, the amount of feed for each pen (column (I) can be easily calculated by dividing the total feed per group (column F) by the number of pens per group (column H). Of course, pen density can be adjusted according to the weight of each group.

Performance Targets

A sound feeding program coupled with professional management should ensure high performance throughout the nursery period. Obviously, sound nutritional, environmental, and disease management are important to the success of an operation. Target weight development in nursery pigs is presented in Figure 1. In Table 6, growth performance targets for a three-phase nursery program are outlined. These levels of performance are currently achieved in many successful commercial nursery units.

Table 1 Specifications for a three-phase feeding program for early-weaned pigs

Item	*Phase I (6 to 8 kg)*	*Phase II (8 to 14 kg)*	*Phase III (14 to 24 kg)*
Ingredients, %			
Corn/wheat	no limit	no limit	no limit
Soybean meal	15 – 25	20 – 30	no limit
Dried whey	20 – 30	10 – 20	0 – 10
Plasma protein	6 – 8	2 – 4	-
Fish meal	0 – 8	0 – 5	0 – 5
Fat or oil	3 – 6	3 – 6	2 – 6
Salt	0.5	0.4	0.3
Growth promotant	+	+	+/?
Nutrients			
Digestible energy, kcal/kg	3,600	3,600	3,600
Protein, %	23 – 26	20 – 24	18 – 22
Lysine, %	1.5 – 1.7	1.3 – 1.5	1.1 – 1.3
True ileal digestible lysine, %	1.2 – 1.3	1.1 – 1.2	1.0 – 1.1
Calcium, %	0.85 – 0.95	0.8 – 0.9	0.7 – 0.8
Phosphorus, %	0.7 – 0.8	0.65 – 0.75	0.6 – 0.7
Available phosphorus, %	0.45 – 0.55	0.4 – 0.5	0.3 – 0.4
Zinc (from ZnO), mg/kg	3,000	2,000	-
Copper (from $CuSO_4$), mg/kg	-	-	150 – 250
Lactose, %	25 – 30	10 – 20	0 – 5
Physical characteristics			
Corn particle size, µm	< 600	< 600	< 600
Diet particle size, µm	< 800	< 800	< 800
Pellet diameter, mm	4 – 6	4 – 6 or meal	4 – 6 or meal

Table 2 Ideal amino acid profile for nursery pig diets[a]

Amino acids	*Relative to lysine (%)*
Arginine	42
Histidine	32
Isoleucine	60
Leucine	100
Lysine	100
Methionine	30
Cystine[b]	30
Phenylalanine	50
Tyrosine[c]	45
Threonine[d]	65
Tryptophan[d]	17
Valine	68

[a]For total, apparent (except for Thr and Trp), and true ileal digestible amino acid concentrations.
[b]Methionine can be used to meet cystine requirements (1 g Met = 0.81 g Cys).
[c]Phenylalanine can cover tyrosine requirements (0.1% Phe = 0.11% Tyr).
[d]Ratios for apparent ileal digestible threonine and tryptophan concentrations are 58 and 15%, respectively.

Table 3 Recommended feed allowances (kg) for early-weaned pigs

	Average weaning weight (kg)				
Diet	*< 4*	*4 – 5*	*5 – 6*	*6 – 7*	*> 7*
Phase I	2.5	2.0	1.5	1.0	0.5
Phase II	8.0	8.0	8.0	8.0	8.0
Phase III	16.0	16.0	16.0	16.0	16.0

Table 4 Distribution of weaning weights with a standard deviation of 1.0 kg

	Average weaning weight (kg)								
Weight (kg)	*4.0*	*4.5*	*5.0*	*5.5*	*6.0*	*6.5*	*7.0*	*7.5*	*8.0*
	Percentage of pigs falling within each weight group								
< 2	2.3								
2 – 3	13.6	6.7	2.3						
3 – 4	34.1	24.2	13.6	6.7	2.3				
4 – 5	34.1	38.3	34.1	24.2	13.6	6.7	2.3		
5 – 6	13.6	24.2	34.1	38.3	34.1	24.2	13.6	6.7	2.3
6 – 7	2.3	6.7	13.6	24.2	34.1	38.3	34.1	24.2	13.6
7 – 8			2.3	6.7	13.6	24.2	34.1	38.3	34.1
8 – 9					2.3	6.7	13.6	24.2	34.1
9 – 10							2.3	6.7	13.6
> 10									2.3

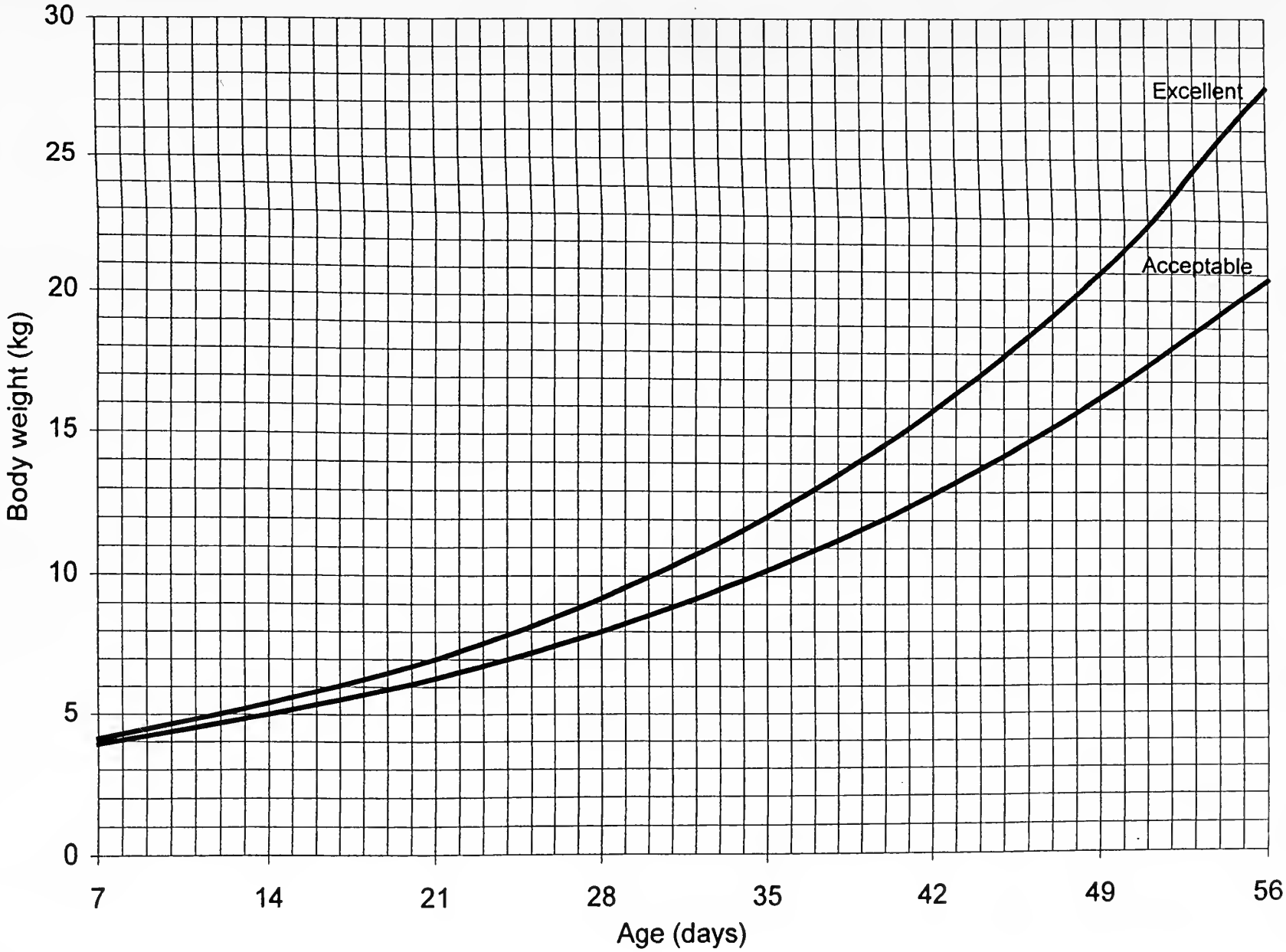

Figure 1 Growth performance targets for nursery pigs in commercial farms

Table 5 Worksheet for Phase I feed budget of a 1,000-head nursery[a]

Weight (kg)	*Pigs*	*Weight groups*	*Pigs/ group*	*Feed/pig (kg)*	*Feed/group (kg)*	*Bags/group (25 kg)*	*Pens of 20 pigs*	*Feed/pen (kg)*
A	B	C	D	E	F	G	H	I
< 4	23							
4 – 5	136	< 5	159	2.0	318	13	8	40
5 – 6	341	5 – 6	341	1.5	512	20	17	30
6 – 7	341	6 – 7	341	1.0	341	14	17	20
7 – 8	136	> 7	159	1.0	80	3	8	10
> 8	23							
Total	1,000		1,000		1,250	50	50	

[a]Average weaning weight of 6 kg and standard deviation of 1 kg.

Table 6 Performance targets for early-weaned pigs

	Duration	*Body weight (kg)*		*Weight gain*	*Feed intake*	*Gain/feed*
Diet	*(d)*	*Initial*	*Final*	*(g/d)*	*(g/d)*	*(g/kg)*
Phase I	7	weaning	8	250	260	960
Phase II	14	8.0	14.0	430	570	750
Phase III	14	14.0	24.0	720	1,150	630
Overall	35			510	740	690

A REVIEW OF IRON NUTRITION IN PIGS

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Man has known that iron plays an important role in health and disease for some considerable time (Loosli, 1978). In fact Bryan (1931) stated that documented therapeutic uses of iron date back as far as 1500 BC. However it was not until Boussingault (1872) that iron was recognized as a vital nutrient for animals. Braasch (1891) was credited with being the first to describe anemia in nursing pigs that were being reared in confinement in Germany. However, he did not equate the anemia with iron deficiency but instead with management. The first to link anemia in nursing pigs with iron deficiency were McGowan and Chrichton (1924). The first to realize this in the United States were Hart et al. (1929) who showed that anemia could be prevented by orally supplementing with ferrous or ferric sulfate. This was after Doyle et al. (1927) had unsuccessfully proposed that baby pig anemia was associated with reduced exposure to sunlight.

Research into bioavailability of iron in feedstuffs is limited due to the fact that anemia is of little significance in other farm species. Iron deficiency is primarily associated with nursing pigs being reared in confinement or animals dependant on milk-based diets. This limits, for practical purposes, the importance of anemia to baby or nursing pigs. Several factors which compound this susceptibility to anemia are: very low iron stores at birth, absence of the polycythemia of birth common to other animals, particularly low levels of iron in sow's milk, and very rapid growth rates compared to other species (Underwood, 1981). In fact, piglets normally quadruple their birth weight by the time they are three-weeks-of age. As mentioned earlier sow's milk is a very poor source of iron, containing only 1 to 3 parts per million (Venn et al., 1947).

The past decade has seen a marked increase in phase feeding of nursery pigs and in the utilization of by-product feed ingredients. Phase feeding is the system of feeding several diets over short periods of time to maximize gain and efficiency. Utilization of by-product ingredients has increased as the feed industry strives to find palatable, highly digestible nutrients which are economically acceptable in animal diets. Research in the area of iron bioavailability for these ingredients has been limited. However, some estimates of iron bioavailability for animal by-products range from 50-60%, with blood meals possibly being higher (Conrad et al., 1980). Hence, there is a need to further investigate the bioavailability of iron in by-product ingredients commonly used in swine diets.

This thesis will evaluate the iron bioavailability of spray-dried blood cells (SDBC) relative to ferrous sulfate in nursery pig diets. The study will consist of two separate hemoglobin (Hb) repletion experiments where hemoglobin repletion will be the criterion used in determining bioavailability.

Iron Compounds in the Body

Iron is a vital component of every living thing. Bothwell et al. (1958) estimated that a 70 kilogram adult human has a whole body iron concentration of 60 to 70 parts per million. The concentration in the pig at birth is approximately 20 to 30 parts per million (Venn et al., 1947). Of this concentration in the newborn pig, 47% is associated with blood, 1.6% is in the spleen, 15% is in liver and the remaining 44% is found in body tissues (Thoren-Tolling, 1975). Following the neonatal period around 80% of the iron in the pig is associated with hemoglobin (National Research Council, 1979). The majority of body iron is bound to proteins as heme complexes or nonheme complexes. The most common heme complexes are hemoglobin and myoglobin, while common nonheme complexes consist of two storage forms, ferritin and hemosiderin, and one transport form, transferrin.

Hemoglobin (Hb) has a molecular weight of 68,000 and consists of four atoms of iron. Hemoglobin is a tetramer consisting of four globin moieties each containing a heme unit bound loosely by noncovalent bonding of iron and the imidazole nitrogen of a histidine residue in each protein chain. The function of hemoglobin is in the transfer of oxygen from the lung to the tissues. Hemoglobin is found in the erythrocytes and makes up roughly 90% of the protein found in those cells (Davies, 1961). In a review, Zimmerman (1980) stated that hemoglobin accounts for 30% of erythrocyte weight. Hemoglobin synthesis, known as hematopoiesis, is carried on in the bone marrow. The average life span of an erythrocyte in swine is 70 days (Bush et al., 1955; Talbot and Swenson, 1963; Jensen et al., 1956; Withrow and Bell, 1969).

Myoglobin differs form hemoglobin in that it contains only one heme group. The molecular weight of myoglobin is 17,000. Myoglobin constitutes only 3 to 7% of total body iron while hemoglobin accounts for about 60% (Hahn et al., 1943). Myoglobin has a much higher affinity for oxygen than that of hemoglobin, this allows for transfer of oxygen from oxyhemoglobin to muscle cells (Fruton and Simmonds, 1958).

Ferritin is a nonneme protein and a primary storage form of body iron that can contain up to 20% iron. The iron contained in ferritin is in the form of ferric oxyhydroxide (Munro, 1977). Ferritin can be found in all tissues of the body, however it is in high concentrations in the liver, spleen, intestinal mucosa and the bone marrow.

Hemosiderin is also a nonheme protein and like ferritin is a storage form of body iron. The iron content of hemosiderin can reach 35% primarily as ferric hydroxide (Shoden and Sturgeon, 1961). Hemosiderin is thought to be produced in times of iron overload.

Transferrin is the transport form of body iron and performs the role of regulating iron distribution within the body. Transferrin like ferritin is transported to all parts of the body but is primarily transported to the liver, spleen, intestinal mucosa, and bone marrow. In swine, transferrin is dependant upon ceruloplasmin for the oxidation of ferrous iron to ferric prior to incorporation into transferrin (Lee et al., 1968; Roeser et al., 1970).

Iron Absorption

Iron absorption can occur thoughout the gastrointestinal tract with the two most common sites of absorption being the duodenum and jejunum. The common theory in iron absorption, known as the mucosal block theory, is that only enough iron to meet the animal's needs is absorbed (Hahn et al., 1943). Two of the main determinants of this need are iron status and erythropoietic demand. This theory has been modified since it's initial statement, however it is still believed that only a small amount of the iron a pig consumes is actually absorbed. The basis of this theory is that iron is taken up by the mucosal cells in one of three forms, ferrous, ferric or as part of an organic compound. Upon absorption ferrous iron is oxidized to the ferric form for incorporation into ferritin. As the mucosal cells become saturated with ferritin, absorption ceases until the ferritin can be converted to transferritin for removal to the plasma. This involves reduction of the iron in ferritin to the ferrous form where it moves to the cell surface and is oxidized before incorporation into transferritin. As ferritin levels in the mucosal cells diminish, iron absorption increases. Therefore, there is an inverse relationship between mucosal ferritin levels and iron absorption. Iron deficient animals absorb dietary iron into the mucosal cells and convert the majority into transferritin while iron adequate animals convert only a small portion of the absorbed iron into transferritin for transport in the plasma (Conrad and Crosby, 1963). Callender et al. (1957) modified the mucosal theory to suggest that heme iron is directly absorbed into the mucosal cells with the porphyrin complex intact.

Factors Affecting Iron Absorption and Bioavailability

There are numerous factors which affect iron absorption and bioavailability such as age, iron status, species, dosage level, and other nutrient components of the diet both organic and inorganic. Furugouri and Kawabata (1976) using labeled ferric citrate showed that newborn pigs exhibited active absorption of iron up to 180 hours of age. Furugouri (1977) postulated that two mechanisms of active transport assist in iron absorption in the neonatal pig, namely endocystosis and absorption of ionic iron across the plasma membrane. Thoren-Tolling (1975) discovered that the neonatal pig is also able to absorb iron bound to macromolecules. Cornelius and Harmon (1973) reported that neonatal pigs are able to absorb considerable amounts of iron dextran via pinocytosis. This confirms previous work by Lecce et al. (1961), who proposed that large organic molecules such as iron dextran should be absorbed intact by pinocytosis. However, Miller et al. (1962) concluded that intestinal changes occur within 30 hours in nursing pigs preventing absorption of these intact macromolecules.

McCance and Widdowson (1937) proposed that efficiency of absorption was due primarily to the iron status of the animal. Bothwell et al. (1958) proposed that the two most important factors concerning iron absorption were iron stores and rate of erythropoiesis. This substantiates the mucosal block theory that an animal only absorbs what it needs or requires. Increasing levels of dietary iron lead to higher total amounts absorbed, however iron status of the animal is still more influential in determining iron absorption (Van Campen, 1974). Excess iron entering the mucosal cells of iron adequate pigs is incorporated in ferritin, only to be lost later in the feces as a product of sloughed mucosal cells (Harmon et al., 1974).

Chausow and Czarnecki-Maulden (1988) noted species differences while completing a hemoglobin repletion study with beef liver and ferrous sulfate using cats and chicks. Iron in beef

liver fed to cats was 350% as available as ferrous sulfate while only 90% as available in chicks. This, however, is not uncommon; if one looks at bioavailability tables there are several differences in bioavailability between species which is illustrated by the study of Pfau et al. (1977). That study showed the bioavailability of hemoglobin in pigs to be 50% relative to ferrous sulfate while Amine et al. (1972) derived a value of 70% using rats and chicks.

Work done with dosage level indicates higher absorption efficiencies with lower dosage levels. Pfau et al. (1977) demonstrated that the absorption efficiency of iron from either hemoglobin or ferrous sulfate to have an inverse relationship to dosage level. As stated previously, Van Campen (1974) reported that even though total amount absorbed may increase with higher doses, the efficiency of absorption decreases.

The physical or chemical form of iron also influences absorption. Iron from animal sources is more available than that from plant sources (Morris, 1987). This is due to the large proportion of heme iron in the animal sources. Heme iron is absorbed as an intact porphyrin complex whereas nonheme iron must be removed from its protein-bound complexes prior to absorption (Morris, 1987). Raffin et al. (1974) reported that once inside the mucosal cell, mucosal heme oxygenase cleaves the heme from the porphyrin ring releasing the iron into the same pathways as that of the nonheme fraction. Wheby and Spyker (1981) concluded this to be the rate limiting step in heme iron absorption from work with iron deficient dogs.

Various nutritive and nonnutritive elements within the diet have also been shown to affect iron absorption and thus, bioavailability. Waddell and Sell (1964) illustrated decreased iron absorption in chicks associated with increasing dietary concentrations of either calcium, phosphorus or both. Phosphorus has been hypothesized to affect iron absorption through the formation of insoluble ferric phosphate and phytate (Underwood, 1981). Bradley et al. (1983) illustrated that dietary Cu concentrations in the range of 120-240 parts per million. led to decreased liver Fe concentrations of 50-60% through possible impairment of iron absorption. Earlier, Gipp et al. (1974) concluded that Cu, when fed in diets at 250 parts per million., not only reduced iron absorption but could invoke iron-deficient anemia in pigs. Gubler et al. (1952) however noted that copper deficiency also hampered iron absorption. Another mineral that shows antagonistic effects on iron utilization is zinc when supplied in excess levels. Settlemire and Matrone (1967a, 1967b) concluded that zinc impacted iron via two methods; by impairment of iron incorporation into ferritin and by decreasing the life span of red blood cells leading to increased iron requirements. Dietary manganese in excessive levels leads to reductions in hemoglobin (Baker and Halpin, 1991).

While the aforementioned minerals have been shown to have detrimental effects on iron absorption and bioavailability, other nutritive factors have been shown to be beneficial. Three of these include the amino acids histidine, lysine and cysteine. Van Campen and Gross (1969) hypothesized that these amino acids form chelates with the ferric iron, thus keeping the iron in solution. These chelates have been identified as tridentate chelates. Vitamin C or ascorbic acid also has been shown to have beneficial effects upon iron absorption. Greenberg et al. (1957) concluded that iron-deficient rats had increased efficiency of absorption of iron when ascorbic acid was given with the iron supplement. This work supports previous findings of Moore and Dubach (1951) who reported increased food iron absorption in man through addition of ascorbic

acid or foods containing ascorbic acid. Van Campen (1972) investigated the effects of histidine and ascorbic acid supplementation on iron absorption and concluded that ascorbic acid was more effective in increasing iron retention. This author hypothesized that this was due to the ability of ascorbic acid to act as both a reducing agent and a chelating agent. Rizk and Clydesdale (1983) meanwhile noted significant decreases in insoluble iron in soy protein isolate following addition of ascorbic acid.

Methods of Determining Bioavailability

Although several methods have been used to measure iron absorption and bioavailability, this review will be limited to hemoglobin repletion and use of radioisotopes. Hemoglobin as a source for quantifying iron bioavailability has been used since early in the 1920's (Mitchell and Schmidt, 1926). Two factors that make hemoglobin an attractive parameter to measure iron bioavailabiltiy are firstly the fact that hemoglobin accounts for roughly 60-80% of the body iron, thus making it a sensitive detector of differing absorption efficiencies, and secondly the relative ease in carrying out hemoglobin determinations. Within the hemoglobin repletion procedure, two different approaches have been used. Pla and Fritz (1970) used the methodology of supplementing an iron deficient diet with graded levels of an iron standard such as ferrous sulfate for creation of a standard curve. Bioavailability of test ingredients would then be determined by supplementation of the test ingredient to the basal diet. Relative bioavailability values were determined through the ratio of hemoglobin gain from the test source to that of the standard. The other common approach used in hemoglobin repletion studies was to compare the slope ratios of hemoglobin response from both the test and standard sources. Amine et al. (1972) developed this method in which graded levels of both the test sources and the standard were supplemented to a common iron deficient basal diet. Advantages of the hemoglobin repletion procedure are that numerous iron sources can be evaluated in a relatively short period of time and that it takes into account both absorption and utilization of the iron; however, the values derived are relative not absolute.

As stated by Smith (1983), methodologies utilizing radioactive tracers, especially extrinsic tagging, for the determination of nutrient bioavailability have become the most popular due to convenience. The overriding principle behind intrinsic tagging is that when a known quantity of radio-iron is added to the food or test ingredient complete isotope exchange occurs. Formation of a homogenous pool, labeled and nonlabeled, of nonheme iron is critical to the resulting bioavailabilities if validity is to be assured (Smith, 1983). The radioactivity is then measured as the response parameter and expressed as a percentage of total iron content. However, in the presence of incomplete isotope exchange the values derived from radiotracers can be overestimates (Van Campen, 1983).

Deficiency Symptoms

Iron-deficient anemia, termed hypochromic-microcytic anemia is generally associated with young, rapidly growing animals deprived of iron in their diet or from their environment. The most common parameter to indicate Fe-deficient anemia is hemoglobin concentration. The National Research Council (1979) created a classification system in which pigs could be categorized to the extent of the anemia by their hemoglobin concentration, measured in

grams/deciliter. Pigs exhibiting hemoglobin levels of ten or above are classified as normal, nine is the minimum level for optimum performance, eight indicates borderline anemia, seven is the level in which anemia retards growth rate, six is considered severe anemia and four as severe anemia with increased mortality. The first signs of iron-deficient anemia is often roughness of haircoat and loss of pigmentation or color of mucous membranes. The skin can become wrinkled and the pigs emit a general listlessness, characterized by drooping of the head and ears combined with a loss of appetite. Iron-deficient anemia was shown to reduce weight gains in affected pigs as early as the 1930's (Moe et al., 1935). In severe cases pigs may be characterized or identified by labored breathing, increased heart and respiratory rates, and even systolic murmurs due to reduced blood viscosity. The largest, fastest growing pigs are susceptible to sudden death from anoxia. Affected pigs have a higher prevalence for subcutaneous edema in the neck, shoulder and limb areas (Conrad et al., 1980). Osborne and Davis (1968) noted that anemic pigs showed higher susceptibility to infectious diseases. This coincides with later research by Nalder et al. (1972) which showed that dietary iron level was directly related to antibody production in weanling rats. Furthermore, Luke and Gordon (1950) showed evidence that anemic pigs were more susceptible to pneumonia, influenza and disorders of the alimentary tract.

Iron Requirements

The definition for net requirement of iron (Underwood, 1981) is the sum of the amounts laid down in the blood and tissues in the process of growth and the amounts lost in feces, urine, sweat, blood loss, at parturition, and in milk and eggs. The newborn pig contains approximately 50 milligram of iron at birth, mostly in the form of hemoglobin (Venn et al., 1947). The neonatal pig has been determined to have a requirement of 7 to 16 milligram of iron per day for normal growth (Venn et al, 1947). Another way in which this can be expressed is a need of 21 milligram of iron per kilogram of weight gain (Braude et al., 1962). Due to the minimal concentration of iron in sow's milk (1 milligram per liter) neonatal pigs reared in confinement require supplemental iron in order to overcome the susceptibility to anemia (Brady et al., 1978). Pigs not supplemented with iron while dependent on sows milk quickly develop iron-deficient anemia (Venn et al., 1947). Maximum hemoglobin levels were produced in neonate at 14 days of age when supplemented with either 100 or 150 milligram of iron dextran at birth (Wahlstrom and Juhl, 1960). Maximum growth rate was acquired through supplementation of 100 milligram in the from of injectable iron dextran to pigs weaned at three weeks of age (Zimmerman et al., 1959). Iron requirement as a concentration of diet decreases with age and weight due to a decrease in blood volume per unit weight and higher iron intakes. The iron requirements for pigs 1 to 5 and 20 to 50 kilogram live weight are 100 and 60 parts per million respectively, which is equivalent to iron intakes of 25 and 114 milligram (National Research Council, 1988).

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APPROACHES TO COLLECTING ILEAL DIGESTA FROM SWINE

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Introduction

Digestibility measurements of feedstuffs are used to determine feedstuff quality and nutritive value. Information on protein and amino acid digestibility is very important for diet formulation because pigs require essential amino acids (AA) for growth. Also, protein is usually the most expensive component of swine diets. Digestibility measurements are reported as either "true" or "apparent". "True" digestibility accounts for endogenous protein, or non-dietary protein that is provided by the digestive tract or digestive secretions. "Apparent" digestibility does not account for endogenous protein, but it is much easier to measure and gives representative information on digestion (Pond et al., 1995).

$$\text{Apparent Digestibility (\%)} = \frac{(\text{AA in feed} - \text{AA in digestive contents})}{(\text{AA in feed})} \times 100$$

$$\text{True Digestibility (\%)} = \frac{\{\text{AA in feed} - (\text{AA in digestive contents} - \text{endogenous AA})\}}{\{\text{AA in feed}\}} \times 100$$

Due to microbial metabolism (Sauer and de Lange, 1992) and the absence of AA absorption in the hindgut (Zebrowska, 1973), the digestive contents (digesta) are routinely sampled from the end of the small intestine (ileum) for digestibility determination. Six different procedures exist to collect ileal digesta from pigs. All of the procedures have positive and negative attributes. It is important to understand these attributes when using digestibility data obtained with different procedures. This report describes the different procedures for ileal collection and offers comparison work that has been conducted.

Collection Techniques

The slaughter method (Low, 1977) involves killing pigs either by electric shock or overdose of anesthesia. The pigs are then dissected, and the ileal contents are removed for analysis. This is a relatively simple procedure, but it is not commonly used. Problems with this procedure include cost and animal-to-animal variation, both of which result from using large numbers of animals. Others problems result from slaughter. When animals are killed, the intestinal contents could shift. Thus, ileal digesta may be contaminated with contents from other parts of the digestive tract. Also, intestinal cell shedding during slaughter confounds protein and AA digestibility measures. However, digestibility work in New Zealand often uses this procedure due to animal welfare concerns.

Simple-T cannulation (Sauer and de Lange, 1992) is a widely used method in digestibility research. This procedure involves the surgical insertion of a "T" cannula into the ileum. Diets fed to simple-T cannulated pigs include a non-absorbed marker that is used to determine digestibility. This is done because the total digestive contents are not collected with this procedure. Digestibility with this technique is determined as follows:

$$\text{Apparent Digestibility (\%)} = 100 - \left(100 \times \frac{\%\text{Marker in feed}}{\%\text{Marker in digesta}} \times \frac{\%\text{AA in digesta}}{\%\text{AA in feed}}\right)$$

This procedure allows multiple collection from the same pig. Many different diets can be fed without blockage problems. During this procedure, the digestive tract is maintained in a normal state because the small intestine is not cut. However, digestibility values obtained from simple-T cannulated pigs could be flawed. Since the total digestive contents are not collected, this procedure relies on the marker behaving as the diet does in the digestive tract. Sometimes, this is not the case. It is important to keep in mind, however, that with proper care and management of the pigs and proper diet mixing, these problems can be minimized. Therefore, this procedure is used extensively in digestibility research.

Re-entrant cannulation (Sauer and de Lange, 1992) is a procedure that involves diverting the entire flow of digesta outside the body, where collection occurs, and then returning the digesta to some part of the digestive tract. This procedure does allow total collection of digesta and multiple sampling from the same pig. However, the use of this procedure is limited for many reasons. First, the small intestine is cut during this procedure, and thus, a normal physiological state is not maintained in the digestive tract. Also, cannula blockage occurs with high-fiber and large-particle diets. When this occurs, feed intake abruptly halts. The re-entrant cannulation procedure is not commonly used.

The ileo-rectal anastomosis (IRA) procedure (Sauer and de Lange, 1992) was developed, and is used commonly, in France. The IRA procedure involves the removal of the hindgut from the digestive tract and the surgical attachment of the ileum to the rectum. Digesta is then collected from the anus. The hindgut is sealed and left inside the pig. Gas build-up is prevented with the use of a T-cannula. This procedure allows for easy collection of any type of diet. A normal digestive state is not maintained with this procedure. Also, intestinal adaptation has been shown to occur with this procedure. Kohler et al. (1992) showed a great increase in volatile fatty acid (VFA) concentrations in the digesta of IRA pigs (See Figure 1). Volatile fatty acid levels indicate microbial activity. There are several hundred species of microbes in the hindgut of pigs. Thus, it has been suggested that the digestive tract adapts to its new state when the IRA procedure is performed. However, this may not accurately reflect what is occurring in the digestive tract.

Postvalvular T-cecum cannulation (van Leeuwen et al., 1988) is a recently developed procedure that involves the insertion of a T-cannula into the cecum. A valve used with this procedure allows collection to occur or allows digesta to proceed into the hindgut. The total contents are collected with this procedure, and many different diets have been tested with this procedure without blockage problems. However, removal of the cecum may alter digestibility

measurements with this procedure. Initial research has indicated that this procedure provides accurate results.

Steered ileo-cecal valve cannulation (Mroz et al., 1996) is the newest collection technique. With this procedure, a cannula is inserted in the large intestine. A metal ring is inserted and secured in the ileum, and a nylon cord is attached to the metal ring. During periods of digesta collection, the nylon cord is pulled to allow the small intestinal contents to exit the cannula. The cord is released during times of non-collection. This allows the digesta to proceed into the hindgut normally. This procedure allows the total contents to be collected, and the small intestine is not cut. However, intestinal adhesions that sometimes form at the site of the metal ring could alter digestibility results. Because this procedure was recently developed, little research has been conducted on its accuracy.

Comparison Research

Only a few studies have been conducted comparing the different procedures. Zebrowska et al. (1978) compared simple-T and re-entrant cannulation procedures. Amino acid digestibility was measured with the semi-purified diets, and few significant differences in digestibility were reported for the two techniques (See Figure 2). Yin et al. (1991) also compared simple-T and re-entrant procedures. It was reported that crude protein digestibility values with simple-T were somewhat higher ($P > 0.05$) than values obtained with re-entrant procedures (See Figure 3). The pigs on this trial were provided commercial-type diets. However, Kohler et al. (1990) compared simple-T, re-entrant and PVTC collection procedures (See Figure 4), and found no differences for the different techniques ($P > 0.05$) in nitrogen digestibility (which is the same as crude protein digestibility) Pigs on this trial were fed commercial-type and high-fiber diets. This discrepancy is possibly due to management and care of the cannulation sites and animals. Another comparison by Leterme et al. (1990) looked at simple-T and IRA techniques and found few significant differences between the procedures (See Figure 5) in the short-term. Pigs on this trial were provided semi-purified diets.

Conclusions

Determining AA and protein digestibility in different feedstuffs is very important for diet formulation in swine production. For this purpose, six different methods exist to collect ileal digesta from pigs. Simple-T cannulation is the most widely used procedure. It provides accurate results when care and management of the pigs is taken into consideration. It is important to consider the collection method used when formulating diets because of the potential problems that exist with each procedure. Further research in needed to compare these methods. What is the best procedure for determining protein and amino acid digestibility? Determining which technique is best depends on personal experience, duration of the experiment, type of diet fed, and animal welfare concerns.

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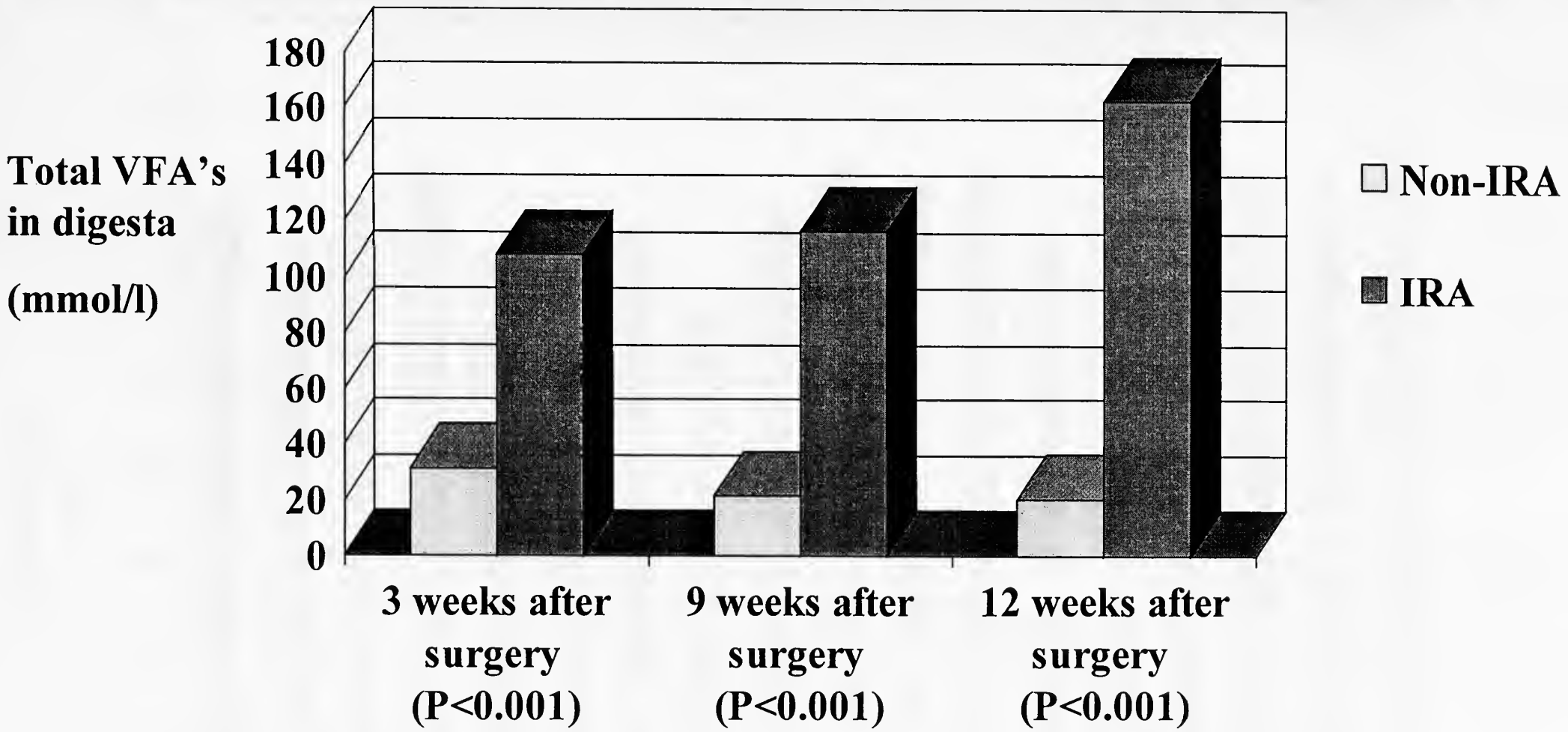

Figure 1: Intestinal adaptation with IRA

Kohler et. al., 1992

P > 0.05 for all amino acids except ASP, TYR and PHE (P < 0.05) and TRP and THR (P < 0.01)

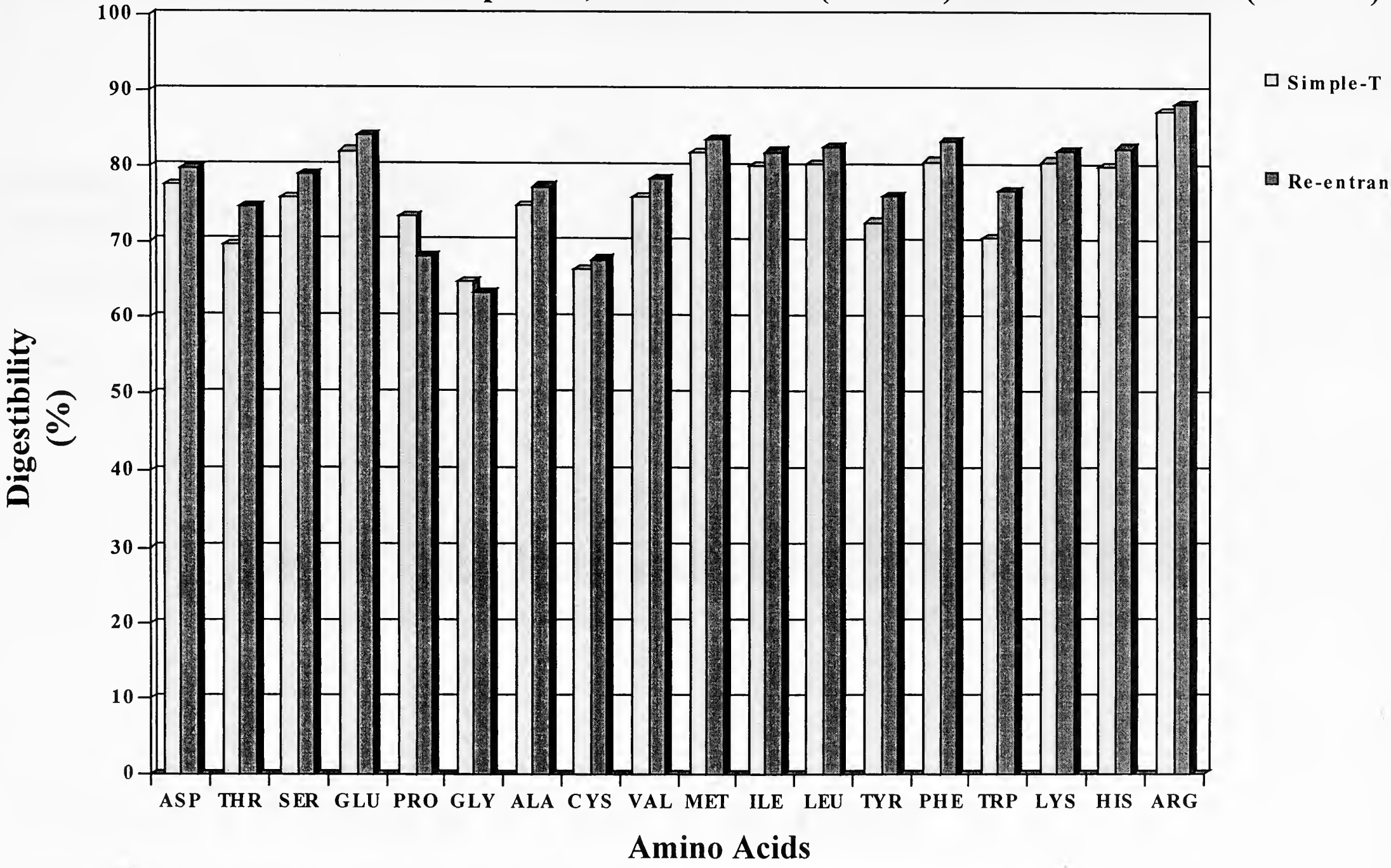

Figure 2: Simple-T vs. re-entrant

Zebrowska et al., 1978

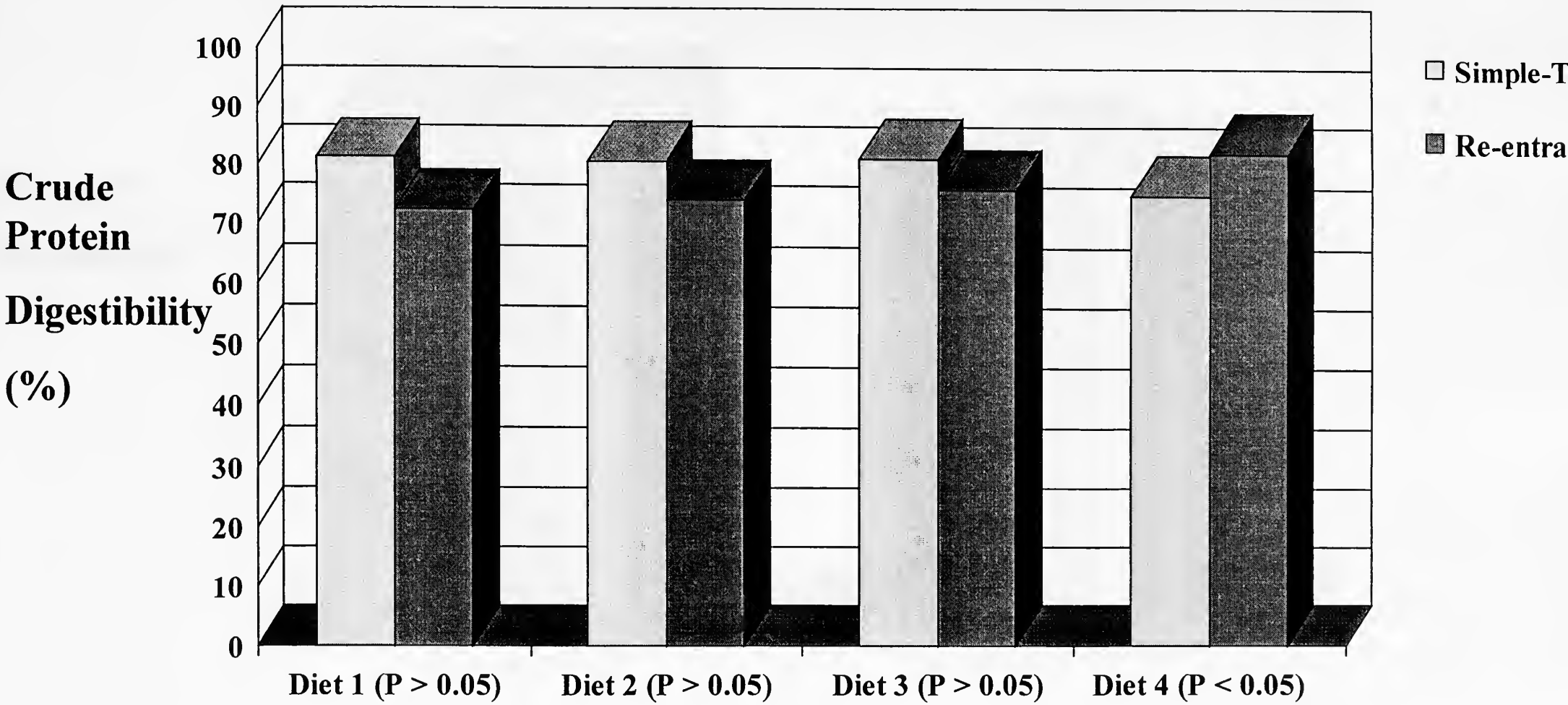

Figure 3: Simple-T vs. re-entrant

Yin et. al., 1991

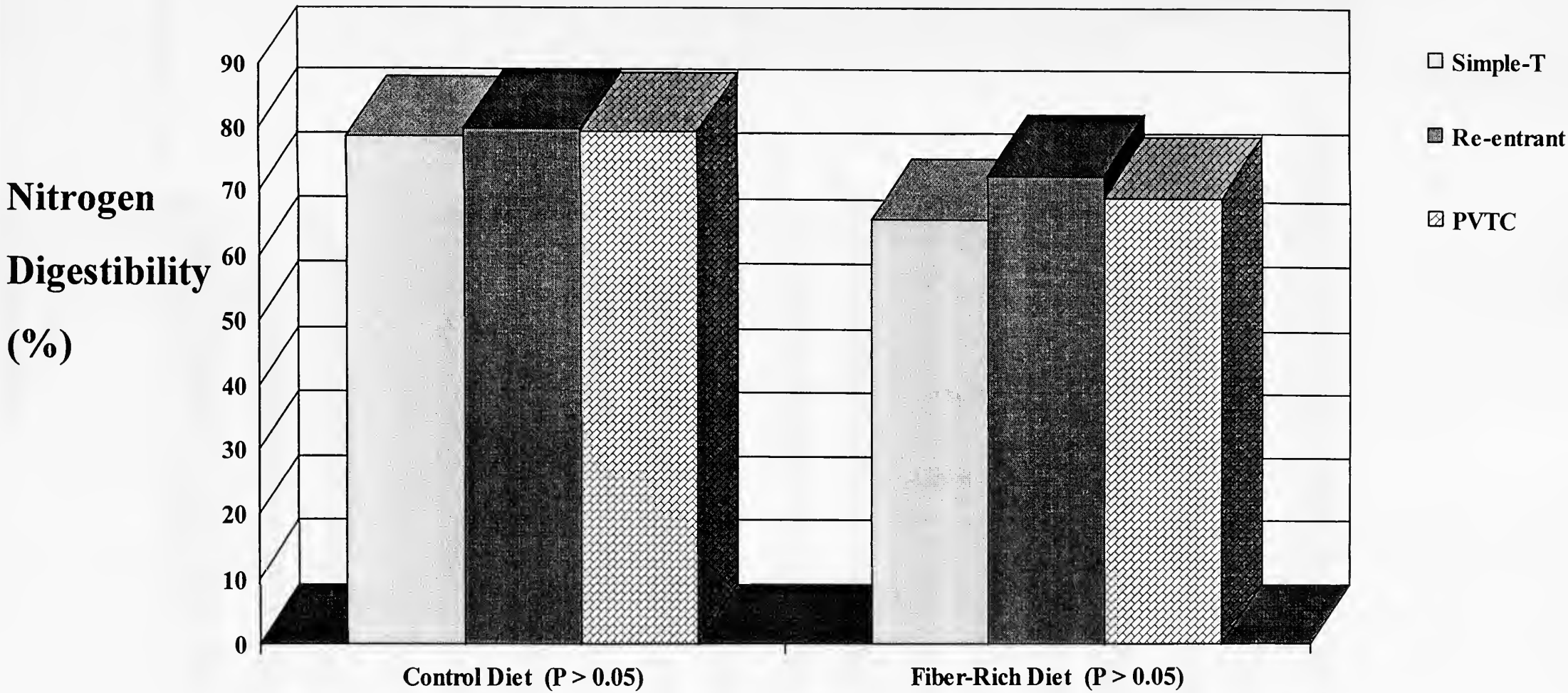

Figure 4: Simple-T, re-entrant & PVTC

Kohler et. al., 1990

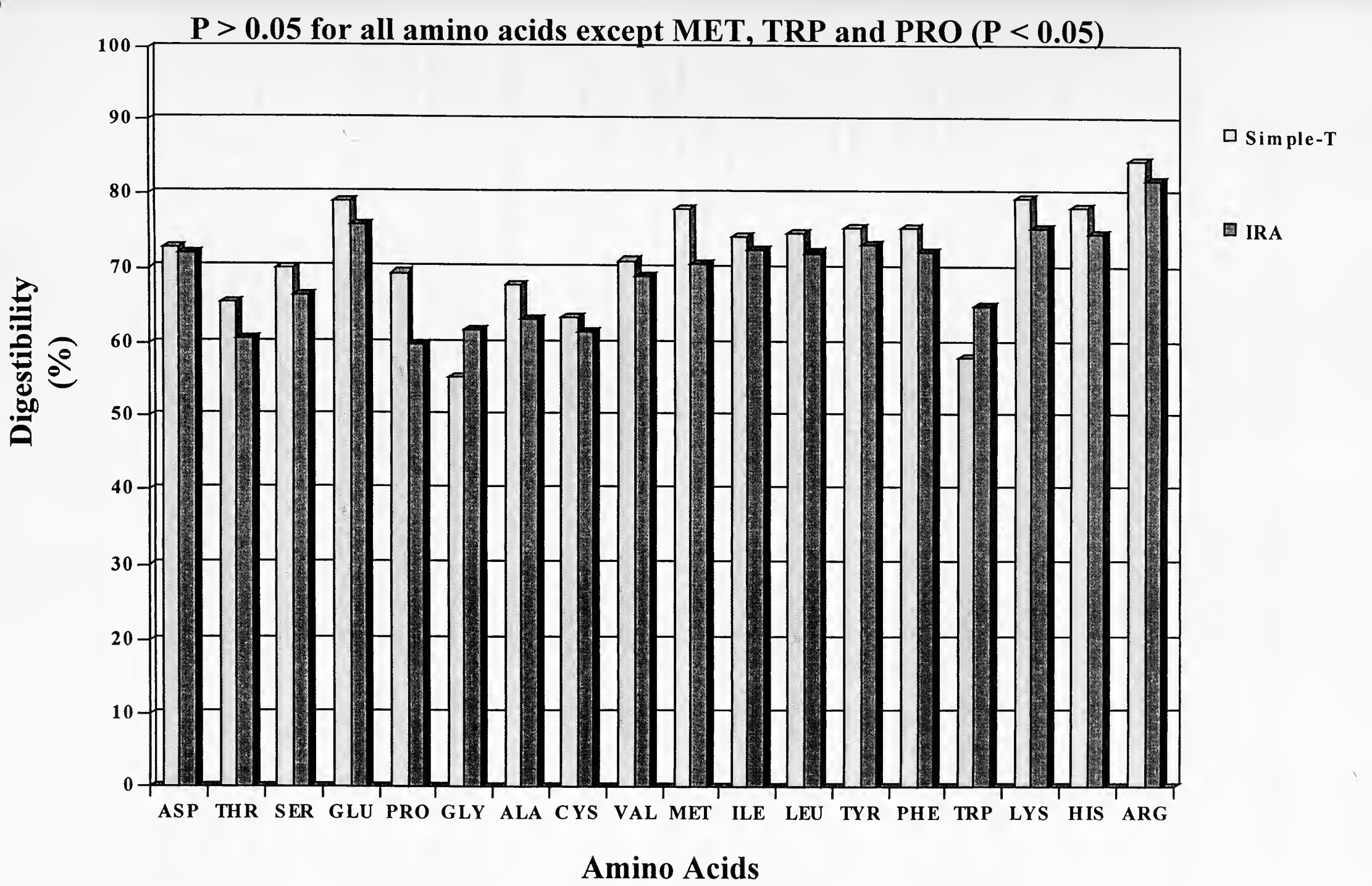

Figure 5: Simple-T vs. IRA

Leterme et al., 1990

INFLUENCE OF FEEDING TANNIC ACID ON BARROWS

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Introduction

Legumes are rich in proteins, as well as being good sources of dietary minerals, vitamins, and calories (Salunkhe et al., 1990). One concern with feeding legumes involves the tannin content of different species and varieties. Tannins are found in many feed sources, including cereal grains, such as barley and sorghum, legumes including soybeans and faba beans, and forages (Mehansho et al., 1987; Salunkhe et al., 1990). Tannins are naturally occurring polyphenolic compounds that have the ability to precipitate proteins (Mehansho et al., 1987).

High tannin diets fed to rats and mice cause hypertrophy of salivary glands and increase the secretion of proline-rich proteins (PRPs) into saliva (Mehansho et al., 1983; Mehansho et al, 1985). The PRPs contain up to 45% proline and have very high binding affinities for tannins (Mehansho et al., 1987). The PRPs are thought to play an important role in neutralizing the negative effects of tannins on protein digestion. Tannins consumed in feeds can cause decreases in feed intake, growth rate, feed efficiency, net and metabolizable energy, and protein digestibility (Mehansho et al., 1983; Mehansho et al., 1985; Jansman et al., 1994). The effect of tannins may occur by binding of tannins to dietary proteins and to intestinal enzymes used in digestion (Reed, 1995).

The objective of this preliminary study was to determine the effects of feeding tannic acid on salivary gland size in young barrows.

Materials and Methods

Twelve barrows (ten weeks of age; bodyweight 32.5 ± 3.5 kg) were randomly divided into two groups and fed a standard diet of corn and soybean meal for the 5 days proceeding the trial. Barrows on control diet received the same standard diet for an additional 5 days, while barrows fed tannic acid (TA) had the same diet containing 2% tannic acid (20g/kg feed) for 5 days. Feed and water were provided ad libitum. Barrows in the TA group received an average of 22 grams of tannic acid per day. Barrows were killed the eleventh day and salivary glands collected.

Result

Parotid glands of the TA barrows dramatically increased in weight ($P < 0.01$; Table 1). In contrast, the submandibular glands were not significantly different between the groups. In studies using rats, the consumption of high tannin sorghums or high tannin faba-bean hulls increased the weight of parotid glands (Jansman et al., 1994). Additionally, rats fed high tannin diets had an increase in the synthesis and secretion of PRPs with a high affinity for binding

tannins (Mehansho et al., 1983; Jansman et al., 1994). Although we have not measured PRPs, the enlarged parotid salivary glands might result in higher secretion of PRPs into saliva in the tannic acid fed barrows compared with control barrows. We are in the process of isolating the PRPs from these salivary glands, as well as determining histological changes in salivary tissue that may be associated with the rapid tissue growth induced by feeding tannic acid.

Conclusion

Addition of tannic acid to diets induced increased parotid gland weights by more than 4-fold during 5 days of feeding tannic acid. These preliminary observations need to be studied further to characterize the effects of dietary tannic acid on feed intake and body weight responses of growing barrows. Changes seen in the salivary glands in response to feeding tannic acid need to be related to any changes in daily feed intake or growth of swine. If the dramatically increased salivary weights induced by feeding tannic acid are paralleled by increased production of salivary PRPs, then high levels of PRPs in saliva may aid in overcoming the potentially negative dietary effects of tannic acid. It remains to be determined if the tannin-neutralizing PRPs are also induced by tannic acid in the pig.

Table 1. Weight of right salivary gland[1]

Salivary gland	*Control diet*	*Tannic acid diet*
Parotid[2]	14.2 ± 4.6 2	68.7 ± 21.4 2
Submandibular	9.6 ± 0.7	10.5 ± 1.7

[1]Grams wet weight.

[2]Significant difference between Control and TA diets ($p<0.01$). Values are mean ± standard error of the mean; n = 6 for each diet group.

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IRON REQUIREMENT OF 5 TO 10 KILOGRAM PIGS

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Abstract

The objective of this study was to determine the optimal requirement for iron (Fe) in the form of ferrous sulfate for 5 to 10 kilograms pigs. A titration study was designed involving thirty-six pigs from six litters of Line 326 x Camborough 22 (Pig Improvement Company, Franklin, KY) pigs. Pigs were reared to weaning (21 days of age) without supplemental iron. At weaning they were moved to elevated-deck nursery pens and randomly assigned to treatment from outcome groups formed on the basis of litter and weight. Blood samples were obtained by jugular puncture and hemoglobin (Hb) and hematocrit (Ht) concentrations were measured colorimetrically. Diets were based on dried skim milk and corn with the common basal diet containing 27 milligrams per kilogram iron. Experimental diets were formulated from aliquots of the basal diet to have incremental levels of 25, 50, 75, 100, and 125 milligrams per kilogram additional iron via substitution of corn starch with ferrous sulfate. Pigs were given ad libitum access to their assigned diet for three weeks with feed disappearance and weight gain recorded for the period. Blood samples were collected at the end of the three week feeding period and again analyzed for hemoglobin and hematocrit. Final hemoglobin values were used for the determination of the break point or optimum level of supplementation of iron in the form of ferrous sulfate. Results of the breakpoint analysis determined an optimal total dietary iron requirement of 71.4 milligrams/day for pigs in the 5 to 10 kilogram range.

Introduction

This experiment was conducted as a hemoglobin repletion study with the purpose of defining the iron requirement for newly-weaned pigs (5 to 10 kilogram liveweight). This study will precede an iron bioavailability study to insure the incremental levels of supplemental iron used in the second trial are in the range required to obtain valid bioavailabilities. Therefore, this experiment is a critical step in the process of determining iron bioavailabilities.

Materials and Methods

Thirty-six weanling pigs from the mating of PIC Line 326 boars and Camborough 22 dams (Pig Improvement Co., Franklin, KY) with an average body weight of 5.22 ± .90 kilogram were used to determine the optimum requirement for iron in the form of ferrous sulfate.

Pigs were farrowed and reared in farrowing facilities at the University of Illinois Swine Research Center, Champaign, IL. Upon farrowing, six litters were selected for use in this study. Rearing procedures differed from conventional practices in only two aspects: supplemental iron injections were not given at birth and access to creep feed was denied. Pigs were weaned at an average of 20.56 days of age, at which time they were weighed and randomly allotted on the basis of ancestry and weight to a nursery facility for the duration of the study.

Pigs were housed individually in raised deck pens where special attention had been given to the removal of all possible environmental iron sources. Pen dimensions were .61 meters x 1.22 meters giving a space allowance of .74 square meters per pig. Pen work consisted of fiberglass fencing with wooden dividers. The flooring in the pens was plastic-coated wire supported over either plastic or wooden gutters allowing for manure storage and removal. Each pen was equipped with individual one-hole stainless steel feeder and water nipples for ad libitum access to feed and water.

A common basal diet was formulated with 27 milligrams per kilogram iron. Experimental diets were then formulated from aliquots of the basal diet to have incremental levels of additional iron, in the form of ferrous sulfate. The additional 25, 50, 75, 100, and 125 parts per million of iron in the experimental diets were achieved via substitution of corn starch with ferrous sulfate. Composition of basal and experimental diets are given in Table 1.

The first three days of the study were an acclimation period in which pigs were given ad libitum access to the basal diet. Pigs were given ad libitum access to their respective experimental diets from day 4 to completion of the study on day 24. Feed was weighed into the feeders daily with feeder weights taken on day 4 and day 24 of the study for determination of total feed and iron intake, average daily feed intake and average daily iron intake. Pigs were also weighed on day 4 and day 24 for determination of total gain and average daily gain. Upon completion of the study gain:feed ratios were calculated from the above data.

Blood samples were taken on day 1 and day 24 of the study via jugular puncture. Vacuum tubes containing potassium EDTA were used to store blood until it could be transferred to the Clinical Pathology Laboratory at the University of Illinois for hematological analysis. Samples were analyzed for hemoglobin (Hb) concentration and percent hematocrit (Ht) with a Celldyn (Abbot Labs, Chicago, Il).

Means for average daily gain, total gain, average daily feed intake, total feed intake, initial and final values for hemoglobin and hematocrit, gain:feed efficiency, and iron intake were analyzed for significant differences using the PROC GLM procedure of SAS (1990). Hemoglobin concentrations for all test diets were analyzed using the linear regression function of SAS (1990), then a breakpoint was calculated using the PROC NLIN function of SAS (1990).

Results

The least square means for average daily gain, average daily feed intake, gain:feed, average daily iron intake, and initial and final hemoglobin concentrations are presented in Table 2 with significant differences noted in superscript. A graphic representation of the optimal iron requirement or breakpoint by plotting final hemoglobin concentrations with respect to increasing dietary intakes of iron is reported in Figure 1.

Average daily gain showed a direct relationship with dietary iron, with pigs on diets A and B growing significantly slower than those on the other diets, which were similar in this respect (Table 2). There was a trend for daily feed intake to increase as the dietary iron concentration

increased, up to 100 parts per million; however, diet differences in feed intake were not statistically significant (Table 2). Gain:Feed increased with dietary iron concentration with treatments A and B being significantly lower than other treatments, which were similar in this respect (Table 2). Average daily iron intake, a direct result of feed intake and treatment, increased linearly with increasing iron concentrations (Table 2). Initial hemoglobin concentrations were similar across treatments except for diet E which was significantly lower than diets A and B (Table 2). There was a general increase in final hemoglobin concentration as dietary iron increased accordingly, with a peak occurring at 125 parts per million of 9.205 grams/deciliter (Table 2). The iron requirement or breakpoint was calculated through the use of the PROC NLIN function of SAS (1990). Final hemoglobin concentration and average daily iron intake were included in the model statement used for the determination of the breakpoint. The breakpoint was determined to be 71 milligrams/day with a slope of .077 (Figure 1).

Discussion

Final hemoglobin concentration and average daily iron intake were the variables used to determine optimal iron requirement in this determination procedure and the breakpoint was calculated at 71.4 milligrams/day. The slope for this equation was determined to be .077, thus yielding a .077 grams/deciliter increase in hemoglobin concentration for every additional 1 milligram supplemental iron, in the form of ferrous sulfate, provided in the diet until intake levels of 71.4 milligrams/day were attained. This iron requirement is higher than the value of 46 milligrams/day recommended by the National Research Council (1988); however this value is for pigs of normal iron status, while the pigs on this study were purposely made anemic.

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Table 1. Percentage Composition of Diets[1]

Ingredient	*Diet A 27 ppm*	*Diet B 50 ppm*	*Diet C 75 ppm*	*Diet D 100 ppm*	*Diet E 125 ppm*	*Diet F 150 ppm*
Corn	54.5362	54.5362	54.5362	54.5362	54.5362	54.5362
Skim milk, dried	30.0000	30.0000	30.0000	30.0000	30.0000	30.0000
AP 920[2]	5.0000	5.0000	5.0000	5.0000	5.0000	5.0000
Soybean oil	4.0000	4.0000	4.0000	4.0000	4.0000	4.0000
Soybean meal	2.8910	2.8910	2.8910	2.8910	2.8910	2.8910
Limestone	1.1000	1.1000	1.1000	1.1000	1.1000	1.1000
Monosodium	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Salt	.2500	.2500	.2500	.2500	.2500	.2500
Vitamin premix[3]	.2000	.2000	.2000	.2000	.2000	.2000
Mineral premix[4]	.0228	.0228	.0228	.0228	.0228	.0228
Corn starch	1.000	.9874	.9749	.9625	.9500	.9375
Ferrous sulfate	0.000	.0126	..0251	.0375	.0500	.0625
Total	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000

[1] "As-is" basis of calculation

[2] Spray-dried animal plasma (American Protein Corporation, Ames, IA)

[3] Provided per kg of diet: 13200 IU vitamin A, 1320 IU vitamin D_3, 58 IU vitamin E, 6.60 mg vitamin K, 81.30 mcg vitamin B_{12}, 19.625 mg riboflavin, 58.181 mg d-pantothenic acid, 87.115 mg niacin, and 1217.795 mg choline.

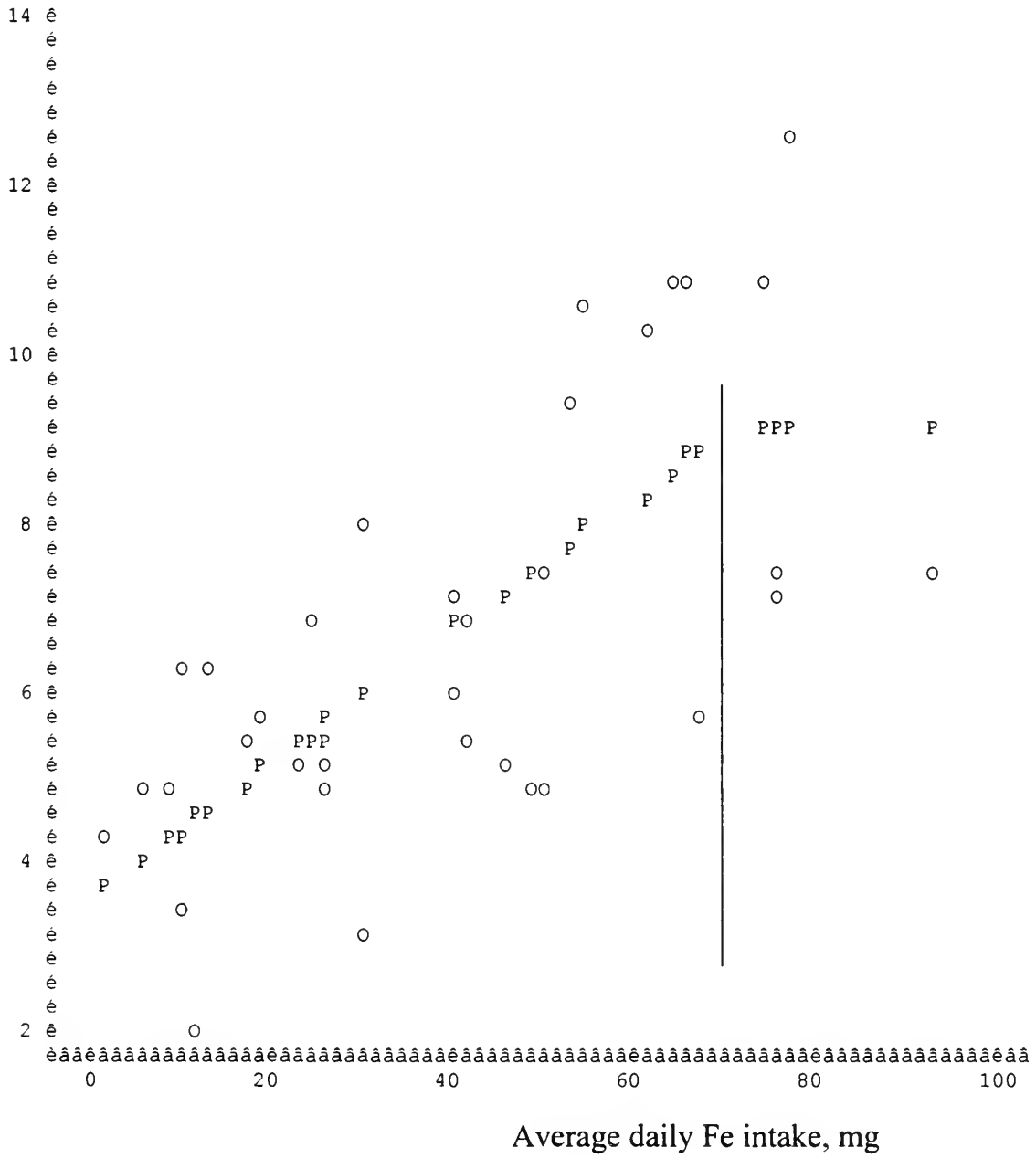
[4] Provided per kg of diet: 10 mg copper (as copper sulfate), .20 mg iodine (as calcium iodide), 20 mg manganese (as manganese oxide), . 30 mg selenium (as sodium selenate), and 100 mg zinc (as zinc oxide).

Table 2. Least square means for response parameters.

Diet *Iron concentration, ppm*	*A* *27*	*B* *50*	*C* *75*	*D* *100*	*E* *125*	*F* *150*	*SEM*
Ave. daily gain, kg	.079[a]	.146[a,b]	.246[b,c]	.246[c]	.260[c]	.282[c]	.341
Ave. daily feed intake, kg	.397[a]	.428[a]	.445[a]	.497[a]	.498[a]	.481[a]	.491
Gain:feed	.144[a]	.324[b]	.550[c]	.489[b.c]	.517[c]	.598[c]	.601
Ave, daily iron intake, mg	10.725[a]	21.409[a.b]	33.052[b]	49.698[c]	62.252[d]	72.124[d]	4.107
Initial hemoglobin, g/dL	5.300[a]	5.112[a]	4.972[a.b]	4.297[a,b]	3.923[b]	4.143[a.b]	.413
Final hemoglobin, g/dL	4.882[a]	5.055[a]	5.711[a.b]	6.920[a.c]	9.205[c]	8.359[b,c]	.867

[a-d]Means in the same row with unlike superscripts differ (P<.05).

Figure 1. Plot of regression and breakpoint analysis of optimal iron requirement of 5 to 10 kg pigs.



BIOAVAILABILITY OF IRON IN SPRAY-DRIED BLOOD CELLS

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Abstract

The objective of this study was to determine the bioavailability, for 5 to 10 kilogram pigs, of iron (Fe) in spray-dried blood cells (SDBC, American Protein Corporation, Ames IA). A slope-ratio design was used involving fifty-six pigs from eleven litters of Line 326 sires x Camborough 22 dams (Pig Improvement Company, Franklin, KY). Pigs were reared to weaning (21 days of age) without supplemental Fe. At weaning they were moved to elevated-deck nursery pens and randomly assigned to treatment from outcome groups formed on the basis of litter and weight. Blood samples were obtained by jugular puncture and hemoglobin (Hb) and hematocrit (Ht) concentrations were measured colorimetrically. Diets were based on dried skim milk and corn with the common basal diet containing 27 milligrams per kilograms total iron. Experimental diets were formulated from aliquots of the basal diet to have incremental additions of 25, 50, and 100 milligrams per kilograms iron via substitution of corn starch with either ferrous sulfate or spray-dried blood cells. Pigs were given ad libitum access to their assigned diet for three weeks with feed disappearance and weight gain recorded for the period. Blood samples were collected at the end of the three-week feeding period and again analyzed for hemoglobin and hematocrit. Final hemoglobin values were plotted and through use of slope ratios the bioavailability of iron in spray-dried blood cells was calculated to be 23.94% that of ferrous sulfate. Statistical analysis showed significant differences among source in regard to final hemoglobin for the highest iron level (3.517 vs 7.436, for diets based on ferrous sulfate and spray-dried blood cells, respectively; $P < .002$). No significant differences were detected between test diets for hematocrit values, however there was a trend toward higher hematocrit values for pigs on ferrous sulfate diets.

Introduction

The use of by-product feed ingredients has been on the increase for the last two decades. Increased use of by-products, especially in nursery pig diets, has been prompted by both the feed industry and producers in an effort to identify those ingredients which contain highly digestible and palatable nutrients which are important for pigs weaned at earlier ages. While a plethora of research has been directed at these ingredients in the past decade, little has been done in the area of iron bioavaibility. Because iron-deficient anemia is more prevalent in humans than livestock, except for the neonatal pig, much of the research in the area of iron bioavailability has centered around foods and sources for human consumption (Morris, 1987). The bioavailability of iron in animal by-products has been estimated to be in the range of 50 to 60% while it is believed that blood products are probably higher (Conrad et al., 1980). However, these are estimates not measured determinations of iron bioavailability. Fritz et al. (1970) reported large variation (8 to

53%) in iron bioavailablity estimates for fish protein concentrates. These variations could be attributed to iron content and processing procedures of the products.
Therefore, this experiment was conducted to determine the iron bioavailability, using a hemoglobin repletion, of a common nursery diet ingredient, spray-dried blood cells, relative to ferrous sulfate.

Materials and Methods

Seventy-two weanling pigs obtained by the mating of PIC Line 326 boars and Camborough 22 dams (Pig Improvement Co., Franklin, KY) with an average body weight of 5.15 ± .89 kilograms were used to determine the bioavailability of iron in spray-dried blood cells (SDBC) in comparison to ferrous sulfate.

Pigs were farrowed and reared in farrowing facilities at the University of Illinois Swine Research Center, Champaign, IL. Upon farrowing, 11 litters were selected for use in this study. Rearing procedures differed from conventional practices in only two aspects: supplemental iron injections were not given at birth and access to creep feed was denied. Pigs were weaned at an average of 21.53 days of age, at which time they were weighed and randomly allotted on the basis of ancestry and weight to a nursery facility for the duration of the study.

Pigs were housed individually in raised deck pens where special attention had been given to the removal of all possible environmental iron sources. Pen dimensions were .61 meters x 1.22 meters giving a space allowance of .74 square meters per pig. Pen work consisted of fiberglass fencing with wooden dividers. The flooring in the pens was plastic-coated wire supported over either plastic or wooden gutters allowing for manure storage and removal. Each pen was equipped with individual one-hole stainless steel feeders and water nipples for ad libitum access to feed and water.

A common basal diet was formulated with 27 milligrams per kilograms iron. Experimental diets were then formulated from aliquots of the basal diet to have incremental levels of additional iron, in either the form of spray-dried- blood cells or ferrous sulfate. The additional 25, 50, and 100 parts per million of iron in the experimental diets were achieved by substitution of corn starch with either product. Composition of basal and experimental diets are given in Table 1.

The first three days of the study were an acclimation period in which pigs were given ad libitum access to the basal diet. Pigs were then given ad libitum access to their respective experimental diets from day 4 to completion of the study on day 24. Feed was weighed into the feeders daily with feeder weights taken on day 4 and day 24 of the study for determination of total feed and iron intake, average daily feed intake and average daily iron intake. Pigs were also weighed on day 4 and day 24 for determination of total gain and average daily gain. Upon completion of the study gain:feed ratios were calculated from the above data.

Blood samples were taken on day 1 and day 24 of the study via jugular puncture. Vacuum tubes containing potassium EDTA were used to store blood until it could be transferred to the Clinical Pathology Laboratory at the University of Illinois for hematological analysis. Samples were

analyzed for hemoglobin (Hb) concentration and percent hematocrit (Ht) with a Celldyn (Abbot Labs, Chicago, Il).

Means for average daily gain, total gain, average daily feed intake, total feed intake, initial and final values for hemoglobin and hematocrit, gain:feed efficiency, and iron intake were analyzed for significant differences using PROC GLM procedure of SAS (1990). Hemoglobin concentrations for both test ingredients were analyzed using the linear regression function of SAS (1990) and compared in a slope-ratio equation.

Results

Least square means for average daily gain, average daily feed intake, feed efficiency, average daily iron intake, initial hemoglobin concentration and final hemoglobin concentration are given in Table 2. A graphic illustration of the regression lines used for the determination of bioavailability by slope ratio of iron in spray-dried blood cells is provided in Figure 1.

Average daily gain increased numerically in response to both supplemental iron sources, though diet G was significantly higher than diets A and E (Table 2). Average daily feed intakes were similar across treatments except for that of pigs fed diet G which was significantly higher than diets A, D, and E (Table 2). There was a general trend for increasing gain:feed as iron concentration increased for the ferrous sulfate supplemented diets; however, this was not the case with the spray-dried blood cell diets (Table 2). Average iron intakes were similar between iron sources for treatments, although diet G had significantly higher iron intakes than all other treatments (Table 2). Pigs on diet B had significantly higher initial hemoglobin concentrations than pigs on the other treatments, which were similar in that respect. Final hemoglobin concentrations, for pigs on the ferrous sulfate supplemented diets, demonstrated a general trend for increasing hemoglobin concentration as dietary iron was increased with pigs fed diet G eliciting significantly higher hemoglobin concentrations than all other diets. Iron bioavaiability was determined using PROC ANOVA function of SAS (1990) and the use of regressions. The slope for the control diet and three ferrous sulfate supplemented diets was .080314. The slope for the control and spray-dried blood cell supplemented diets was .019229. The bioavailability was then determined by dividing the slope of the spray-dried blood cell regression by the slope of the ferrous sulfate regression. This gives a iron bioavailability coefficient of spray-dried blood cells of 23.94% relative to ferrous sulfate.

Discussion

Hemoglobin repletion has been identified as a valid method for determining iron bioavailabilities since the 1920's (Mitchell and Schmidt, 1926). It is a valid response parameter in determining iron bioavailability due to the relatively high proportion of body iron (approximately 60-80%) associated with hemoglobin. This makes hemoglobin a sensitive indicator of iron status in the neonatal pig. This study utilized the repletion method outlined by Amine et al. (1972) in which graded levels of both the standard and test ingredient are added to the a common basal diet and slope ratios calculated. The bioavailability derived in this experiment was 23.94% relative to ferrous sulfate. This value is lower than previous estimates by Conrad et al. (1980) who

estimated iron in animal by-products at 50 to 60% available with blood meals probably higher in bioavailability. However, the estimates of Conrad et al. (1980) were estimations of bioavailability, not absolutes.

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Table 1. Percentage Composition of Diets[1]

Ingredient	*Diet A 27 ppm*	*Diet B 52 ppm*	*Diet C 77 ppm*	*Diet D 127 ppm*	*Diet E 52 ppm*	*Diet F 77 ppm*	*Diet G 127 ppm*
Corn	52.2422	52.2422	52.2422	52.2422	52.2422	52.2422	52.2422
Skim milk, dried	24.1500	24.1500	24.1500	24.1500	24.1500	24.1500	24.1500
AP 920[2]	6.9000	6.9000	6.9000	6.9000	6.9000	6.9000	6.9000
Soybean oil	3.6800	3.6800	3.6800	3.6800	3.6800	3.6800	3.6800
Soybean meal dehulled	2.6590	2.6590	2.6590	2.6590	2.6590	2.6590	2.6590
Limestone	1.0120	1.0120	1.0120	1.0120	1.0120	1.0120	1.0120
Monosodium phosphate	.9200	.9200	.9200	.9200	.9200	.9200	.9200
Salt	.2300	.2300	.2300	.2300	.2300	.2300	.2300
Vitamin premix[3]	.1840	.1840	.1840	.1840	.1840	.1840	.1840
Mineral premix[4]	.0228	.0228	.0228	.0228	.0228	.0228	.0228
Corn starch	8.0000	7.0740	6.1485	4.2962	7.9875	7.9750	7.9500
AP 301[5]	.0000	.9260	1.8515	3.7038	.0000	.0000	.0000
Ferrous sulfate	.0000	.0000	.0000	.0000	.0125	.0250	.0500
Total:	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000

[1] "As-is" basis of calculation

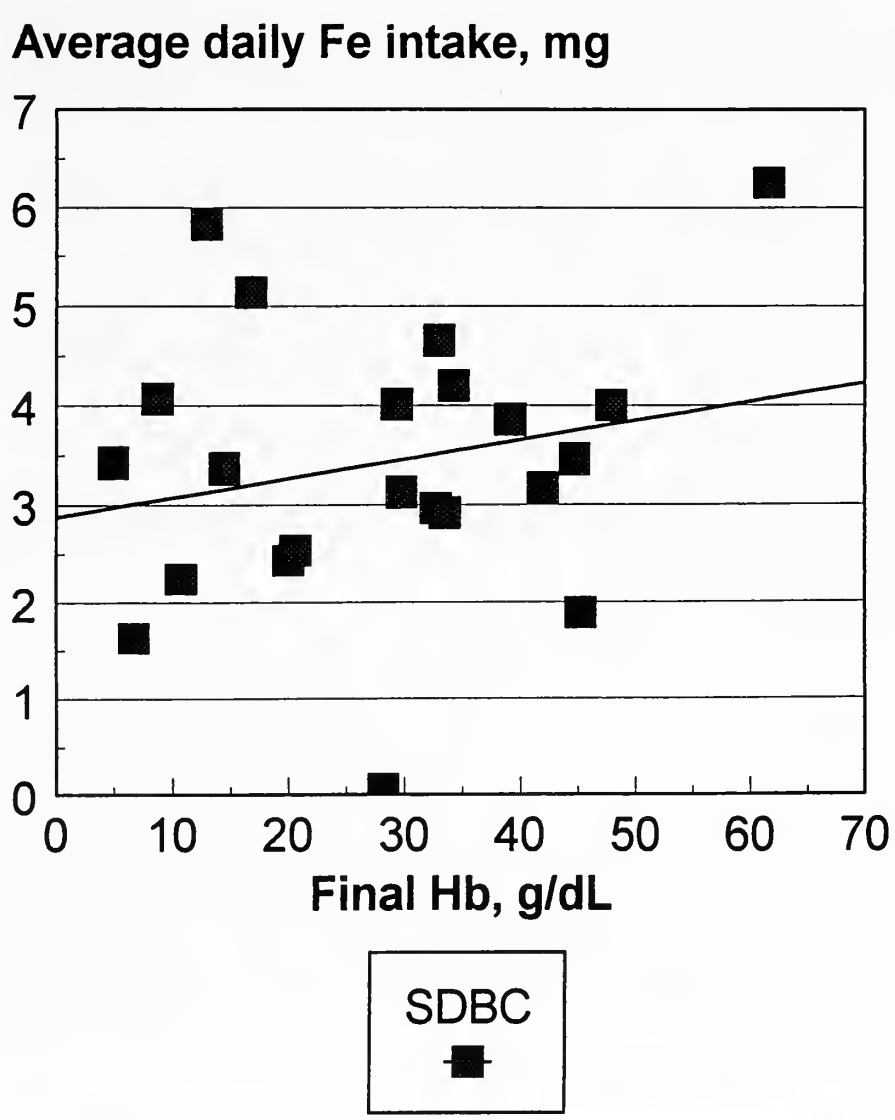
[2] Spray-dried animal plasma (American Protein Corporation, Ames, IA)

[3] Provided per kg of diet: 13200 IU vitamin A, 1320 IU vitamin D_3, 58 IU vitamin E, 6.60 mg vitamin K, 81.30 mcg vitamin B_{12}, 19.625 mg riboflavin, 58.181 mg d-pantothenic acid, 87.115 mg niacin, and 1217.795 mg choline.

[4] Provided per kg of diet: 10 mg copper (as copper sulfate), .20 mg iodine (as calcium iodide), 20 mg manganese (as manganese oxide), .30 mg selenium (as sodium selenate), and 100 mg zinc (as zinc oxide).

[5] Spray-dried animal blood cells (American Protein Corporation, Ames, IA)

Figure 1. Bioavailability regressions for both SDBC and ferrous sulfate.



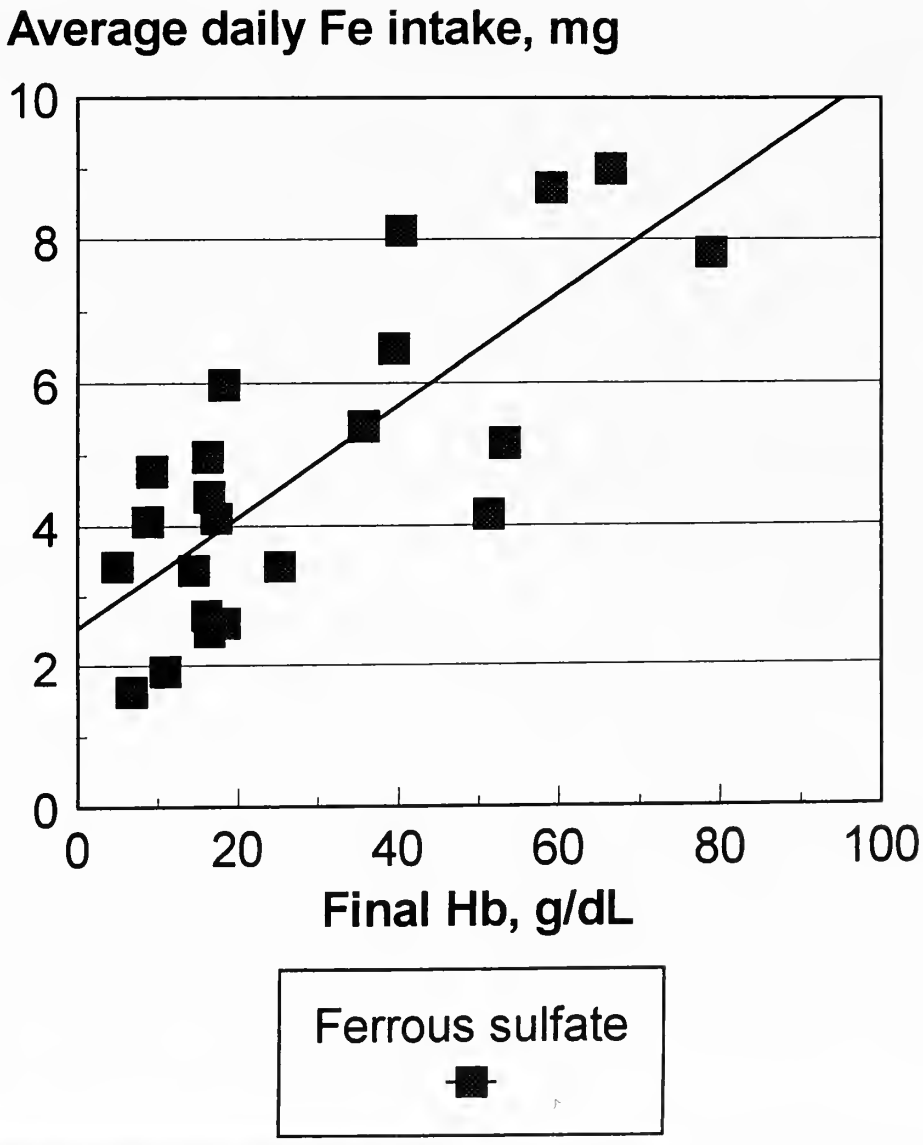


Table 2. Least square means for response parameters of bioavailability study.

Diet *Iron concentration (ppm)*	*A* *27*	*B* *52*	*C* *77*	*D* *127*	*E* *52*	*F* *77*	*G* *127*	*SEM*
Ave. daily gain, kg	-.001[a]	.099[a,b]	.106[a,b]	.115[c,d]	.072[a,b]	.114[b,d]	.234[c,d]	.042
Ave. daily feed intake, kg	.314[a]	.383[a,b]	.411[a,b]	.335[a]	.352[a]	.395[a,b]	.515[b]	.052
Gain:feed	.016[a]	.242[a,b]	.224[a,b]	.351[b]	.180[a,c]	.202[a,b]	.470[b]	.095
Ave. daily iron intake, mg	8.08[a]	19.07[a,b]	30.78[b,c]	42.03[c,d]	17.68[a,b]	29.61[b,d]	64.41[e]	6.27
Initial hemoglobin, g/dL	3.978[a]	4.585[b]	3.938[a,b]	3.549[a]	3.905[a,b]	3.921[a,b]	3.964[a,b]	.370
Final hemoglobin, g/dL	2.993[a]	3.789[a]	3.116[a]	3.460[a]	3.974[a]	4.818[a]	7.357[b]	.695

[a-e]Means in the same row with unlike superscripts differ (P<.05).

VALINE IS LIMITING IN LOW-PROTEIN DIETS FOR NURSERY PIGS

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Introduction

Confusion exists concerning the limiting amino acids and their order of limitation for young growing pigs fed low-protein diets based on corn and soybean meal. The purpose of our study was to clarify the limiting amino acids, and their order, for 10 kg pigs of a high lean-gain genotype that are fed a 13.5% CP corn-soybean meal diet containing 8% dried whey. Three amino acid deletion experiments were carried out involving the essential amino acids Lys, Trp, Thr, Met, Val, Ile, and His, and also Glu as a source of nonspecific amino nitrogen. The positive-control diet was a 19.2% CP corn-soybean meal-based diet (1.15% Lys), also with 8% dried whey. Amino acid additions to the low-protein, negative-control diet were based on levels needed to accomplish 110% of ideal ratios (to Lys, set at 1.15%).

Experiment 1

A total of 108 PIC nursery pigs with an average initial body weight of 8.8 kg and average age of 35 d was used in a 21-d growth assay. Treatment diets included: 1) a corn-soybean meal-based diet (19.2% CP, 1.15% lysine), fortified with 8% dried whey (Table 1); 2) a low protein (13.5% CP, .64% lysine) corn-soybean meal-whey-based diet; 3) the same 13.5% CP diet fortified with crystalline amino acids to reach 110% of ideal amino acid concentrations; and 4 to 9) the 13.5% CP fortified diet with single amino acid deletions (Lys, Trp, Thr, Met, Ile, or Val, respectively). Addition of an amino acid mixture containing Lys, Trp, Thr, Met, Ile, and Val to the low-protein diet increased ($P < .05$) gain and gain:feed ratio, and these response traits were not different from those of pigs fed the 19.2% CP positive-control diet. Single deletion of Lys from the supplemental amino acid mixture depressed performance to a greater ($P < .05$) extent than single deletion of any of the other amino acids. Single deletions of Trp, Thr, Met, or Val decreased ($P < .05$) performance in a similar but lesser magnitude than the decrease caused by Lys deletion, whereas Ile deletion was without effect.

Experiments 2 and 3

Experiments 2 and 3 were designed to evaluate the limiting order of AA beyond Lys in the low-protein diet. In Exp. 2, a total of 360 (Line 326 sire H C22 dams; PIC, Franklin, KY) nursery pigs with an average initial body weight of 9.0 kg and average age of 35 d was used in a 21-d growth assay. Treatments included the same positive control as in Exp. 1. The 13.5% negative-control diet in Exp. 2 (diet 2) contained supplemental Lys and Ile. Diet 3 contained supplemental amino acids (Trp, Thr, Met, Val, His, and Glu) that were considered potentially

limiting after Lys. Each of the amino acids in diet 3 was then individually deleted (diets 4 to 9) such that relative growth depressions could be assessed.

In Exp. 3, a total of 147 (mixed color-line sires x mixed white-line dams) nursery pigs with an average initial body weight of 8.9 kg and average age of 35 d was used in a 21-d growth assay. Treatments included the same positive and negative controls as in Exp. 2. Amino acids under test included Trp, Thr, Met, and Val. Unlike the other two experiments which were conducted at the University of Illinois Swine Research Center, this experiment was conducted in an all-in all-out commercial nursery facility (United Feeds, Gridley, IL). Diet preparation was done at the University of Illinois Swine Research Center using the same ingredients as for Exp. 1 and 2.

Neither His nor Glu were found to be deficient, and as in Exp. 1, Trp, Thr, Met, or Val deletion from the supplemental amino acid mixture resulted in performance depressions ($P < .05$) that were similar.

Implications

Valine was as limiting as tryptophan, threonine, or methionine in a 13.5% protein corn-soybean meal diet for 10-kg pigs. This suggests that the valine requirement of 10-kg pigs may be higher than the National Research Council (1998) has predicted. The data clearly showed that valine was more limiting than isoleucine in a reduced protein corn-soybean meal diet for young pigs.

Table 1. Percentage composition of basal diets (as-fed basis)

Ingredient	*Low-protein negative control*	*High-protein positive control*
Cornstarch	to 100	to 100
Corn	67.24	59.13
Dehulled soybean meal	14.53	26.28
Edible-grade dried whey	8.00	8.00
Degummed soybean oil	3.00	3.00
Dicalcium phosphate	1.20	1.20
Limestone	.80	.80
Trace mineral premix[a]	.35	.35
Vitamin premix[b]	.20	.20
Antibiotic[c]	.50	.50
$CuSO_4 \bullet 5H_2O$	.05	.05
L-Lysine•HCl	-	.28
DL-Methionine	-	.13
L-Threonine	-	.08
Chemical Composition		
Crude protein, %[d]	13.50	19.20
Lysine, %[d]	.64	1.15
Methionine, %[d]	.24	.42
Cystine, %[d]	.25	.33
Threonine, %[d]	.56	.83
Tryptophan, %[d]	.15	.22
Valine, %[d]	.71	.94
Isoleucine, %[d]	.57	.79
Digestible energy, kcal/kg[e]	3,335	3,314

[a]Supplied the following per kilogram of complete diet: Fe, 90 mg ($FeSO_4 \bullet H_2O$); Zn, 100 mg (ZnO); Mn, 20 mg (MnO); Cu, 8 mg (CuO); I, .35 mg (CaI_2); Se, .3 mg (Na_2SeO_3); NaCl, 3 g.

[b]Supplied the following per kilogram of complete diet: retinyl acetate, 2,273 μg; cholecalciferol, 16.5 μg; dl-α-tocopheryl acetate, 88 μg; niacin, 33 μg; d-Ca-pantothenate, 24.2 μg; riboflavin, 8.8 μg; vitamin B_{12}, .035 μg; menadione sodium bisulfite complex, 4.4 μg; choline chloride, 324 mg.

[c]Provided 110 mg of chlortetracycline, 110 mg of sulfamethazine, and 55 mg of penicillin per kilogram of complete diet.

[d]Analyzed (AOAC, 1995).

[e]Calculated (NRC, 1998).

Table 2. Use of a deletion assay to determine the limiting amino acids in a 13.5% crude protein corn-soybean meal-whey diet for nursery pigs (Exp. 1)[a]

Dietary treatment	*Daily weight gain, g*	*Daily feed intake, g*	*Gain:feed, g/kg*
1. 19.2% CP, positive control	554^{w}	1007^{w}	550^{w}
2. 13.5% CP, negative control[b]	387^{z}	942^{wxy}	411^{z}
3. As 2 + Lys, Trp, Thr, Met, Ile, and Val[c]	543^{w}	1044^{w}	520^{wx}
4. As 3 - Lys[c]	419^{yz}	984^{wx}	426^{yz}
5. As 3 - Trp[c]	467^{xy}	843^{xy}	554^{w}
6. As 3 - Thr[c]	483^{x}	1002^{w}	482^{xy}
7. As 3 - Met[c]	476^{x}	979^{wx}	486^{x}
8. As 3 - Ile[c]	535^{w}	1057^{w}	506^{wx}
9. As 3 - Val[c]	406^{z}	814^{y}	501^{wx}
Pooled SEM	17	48	19

[a]Data are means of four pens of three pigs during a 21-d feeding period (35 to 56 d of age); average initial and final body weights were 8.8 and 18.5 kg, respectively.

[b]See Table 1 for composition of basal diet.

[c]Levels added or deleted were: L-Lys HCl, .67%; L-Trp, .06%; L-Thr, .26%; DL-Met, .27%; L-Ile, .17%; L-Val, .18%.

[wxyz]Means within columns followed by different superscript letters are different ($P < .05$).

Table 3. Limiting order of amino acids after lysine in a 13.5% crude protein corn-soybean meal-whey diet for nursery pigs (Exp. 2)[a]

Dietary treatment	*Daily weight gain, g*	*Daily feed intake, g*	*Gain:feed, g/kg*
1. 19.2% CP, positive control	607^{w}	890^{wx}	682^{w}
2. 13.5% CP + Lys + Ile[b]	465^{y}	889^{wxy}	523^{yz}
3. As 2 + Trp, Thr, Met, Val, His, and Glu[c]	538^{x}	881^{wxy}	611^{x}
4. As 3 - Trp[c]	472^{y}	894^{xy}	528^{yz}
5. As 3 - Thr[c]	461^{y}	943^{w}	489^{z}
6. As 3 - Met[c]	458^{y}	856^{xy}	535^{y}
7. As 3 - Val[c]	433^{y}	825^{y}	525^{yz}
8. As 3 - His[c]	520^{x}	855^{xy}	608^{x}
9. As 3 - Glu[c]	530^{x}	875^{xy}	606^{x}
Pooled SEM	15	28	16

[a]Data are means of eight pens of five pigs during a 21-d feeding period (35 to 56 d of age); average initial and final body weights were 9.0 and 19.1 kg, respectively.

[b]Diet as shown in Table 1, but also containing .66% L-Lys HCl and .17% L-Ile.

[c]Levels added or deleted were: L-Trp, .06%; L-Thr, .26%; DL-Met, .27%; L-Val, .18%; L-His, .03%; L-Glu, 2.5%.

[wxyz]Means within columns followed by different superscript letters are different ($P < .05$).

Table 4. Use of a deletion assay to determine the limiting order of amino acids after lysine in a 13.5% crude protein corn-soybean meal-whey diet for nursery pigs (Exp. 3)[a]

Dietary treatment	*Daily weight gain, g*	*Daily feed intake, g*	*Gain:feed, g/kg*
1. 19.2% CP, positive control	533[w]	927[wx]	575[w]
2. 13.5% CP + Lys + Ile[b]	371[y]	881[wx]	421[z]
3. As 2 + Trp, Thr, Met, and Val[c]	546[w]	1013[w]	539[wx]
4. As 3 - Trp[c]	470[wx]	867[wx]	542[wx]
5. As 3 - Thr[c]	453[xy]	960[w]	472[y]
6. As 3 - Met[c]	487[wx]	962[w]	506[xy]
7. As 3 - Val[c]	402[xy]	794[x]	506[xy]
Pooled SEM	31	55	14

[a]Data are means of three pens of seven pigs during a 21-d feeding period (35 to 56 d of age); average initial and final body weights were 8.9 and 18.7 kg, respectively.
[b]Diet as shown in Table 1, but also containing .66% L-Lys HCl and .17% L-Ile.
[c]Levels added or deleted were: L-Trp, .06%; L-Thr, .26%; DL-Met, .27%; L-Val, .18%.
[wxyz]Means within columns followed by different superscript letters are different ($P < .07$).

THE INFLUENCE OF LEVEL OF DIETARY VITAMIN D_3 SUPPLEMENTATION AND POST-MORTEM AGING TIME ON PORK QUALITY

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Introduction

Tenderness is one of the most critical attributes in determining consumer satisfaction with pork. In general, tenderness increases following a 7 to 10 day postmortem aging period (Smith et al., 1978) and this provides a mechanism by which meat can be influenced postmortem to insure tenderness in fresh meat products. Tenderization of meat products is proposed to result from the activities of a family of calcium-dependent proteolytic enzymes present in the muscle, known as calpains. The activities of these enzymes, which are controlled by the calcium concentration in the muscle, result in the breakdown of muscle fibers, resulting in more tender meat.

Vitamin D_3 has been shown to increase the amount of free calcium transported across the intestinal wall. This increase in calcium absorption is the result of increased calcium binding proteins (CBP) present in the intestine. The "free" calcium is taken up by the bloodstream and utilized in various body functions, while the remaining is deposited in the muscle.

Altering calcium ionic concentration in the muscle tissue through marination in calcium chloride has been shown to increase tenderness in beef longimussis muscle. This increase in tenderness by activation of calpain enzymes through addition of calcium has (Whipple and Koohmaraie. 1993) very limited practical application in the commercial packing industry. Other methods of altering calcium ionic strength present in muscle tissue such as feeding high levels of vitamin D_3 to feedlot steers before slaughter has been shown to improve the tenderness of beef (Swantek et al., 1997). The objective of this study was to determine the effects of feeding high levels of vitamin D_3 on growth performance, carcass characteristics, meat quality, and palatability traits in pigs. In addition, the impact of postmortem aging on tenderness was investigated.

Experimental Approach

Trial Design and Performance Test. The study was conducted at the Moorman Swine Research Farm at the University of Illinois over a ten day period prior to slaughter. A total of thirty-six Yorkshire x Duroc and purebred Duroc pigs (average initial body weight 105 $\pm$ 1.4 kg) comprising of equal number of barrows and gilts were allotted to groups of six on the basis of weight, sex, and genotype. Pigs were housed in conventional accommodation and subjected to standard commercial management until allocated for test. Groups of pigs were randomly allotted to one of three dietary vitamin D_3 levels as follows:1) 331 IU/kg of feed (L); 2) 55031 IU/kg of feed(M); and 3) 176,000 IU/kg of feed(H). Diets were formulated to exceed calcium requirements for finishing pigs by 15% and to meet or exceed requirements for other nutrients

based on NRC (1988). During the study pigs were housed in a controlled environment finishing house which had total slatted flooring and provided with a pen space allocation of 1.2 m /pig. Pigs were given ad libitum access to feed from a two-hole feeder and had free access to water. Blood samples for serum calcium determination were taken at day 0 to establish baseline concentrations and on day 10 to monitor the serum calcium response. Pig weights were recorded at the start and end of the feeding period and group feed intakes were monitored over the feeding period. At the conclusion of the feeding period, pigs remained in their test groups on full feed until they were dispatched from the farm on the morning of slaughter and transported to the Meat Science Laboratory at the University of Illinois where they were held for approximately 1 hour before slaughter. Pigs were mixed with those from other groups during transport and in lairage where they were held with access to water but not feed.

Slaughter and Carcass Evaluation. Pigs were weighed immediately before slaughter, which was carried out using commercial slaughtering practices with viscera (including liver and kidneys) being destroyed. Following evisceration, carcasses were split down the midline and hot carcass weights were recorded approximately 1 hour postmortem after which carcass were placed in a chiller (4° C) where they were held overnight. At 24 hour postmortem, cold carcass weights were recorded and carcass measurements were taken on the left side of each carcass. Carcass measurements included the following: carcass length (measured from the cranial tip of the aitch bone to the cranial edge of the first rib adjacent to the thoracic vertebra); midline fat measurements (opposite the 1^{st} rib,10^{th} rib, last rib, last lumbar vertebra); longissimus muscle area (measured using a dot grid) and depth at 6.5 cm off the midline at the 10^{th} rib.

Meat Quality analysis. Meat quality assessments were made on the loin muscle from the left side of the carcass. Loins were boned and a muscle section (from 10^{th} rib to last lumbar) was used for subsequent analysis. Measurements included 24 hour pH, subjective scores for loin color, firmness, and marbling on the cut surface of the muscle immediately posterior to the last rib using the procedures described by NPPC (1991) . Color, firmness, and marbling were evaluated using a 5-point scales (1= pale, soft, and devoid of marbling; 5= dark, firm, and moderately abundant or greater marbling). A loin chop (1.0 cm thick) was obtained from the posterior end of each loin section for drip loss determination. The chop was weighed, placed in a Whirlpak® bag, suspended in a 4°C cooler for 24 h, and then re-weighed, and drip loss was determined. The remaining portion of the loin muscle was broken into two equal sections. Loin objective color was determined using a Hunter Labscan II colorimeter. Color scores (L*, a*, and b* values represent lightness, redness, and yellowness, respectively) were obtained on the cut surface of each section. The loin sections were then randomly assigned to one of two aging treatments of 7 or 14 days. Samples were vacuum-packaged, then stored in a 4°C cooler according to aging treatment. After aging, loin sections were frozen (-20°C) for subsequent use in sensory evaluation and Warner-Bratzler shear force determination.

Shear Force and Sensory Evaluation. Frozen loin sections were cut into chops (2.5 cm thick) on a band saw. Samples were thawed for 24 h at 4° C for Warner-Bratzler shear force evaluation. The chops were cooked on Faberware open hearth grills to an internal temperature of 70°C with temperature being monitored using copper Constantan thermocouples and a recording

thermometer. Chops were weighed before and after cooking to determine cooking loss. Following cooling to 25°C, four 1.3-cm diameter cores were removed from each chop parallel to the direction of the meat fibers and sheared using an Instron universal testing machine fitted with a Warner-Bratzler shear attachment. The full scale load was set at 10 kg, and the chart with the average sheer force of the cores being representable of sample. Chops for sensory evaluation were prepared and cooked using the same procedures as for shear force. Ten panelists consisting of faculty and graduate students at the Meat Science Laboratory were trained according to the procedures for sensory evaluation described by the American Meat Science Association (1978). A taste panel consisting of six trained panelists evaluated tenderness, juiciness, and off-flavor intensity using a 15 cm structured line scale with anchors and a midpoint (0 cm = extremely dry, tough, and intense off-flavor; and 15 cm = extremely moist, tender, and no off-flavor). Water was provided to the panelists to cleanse the palate.

Results

The growth performance and serum calcium levels are presented in Table 2. Serum calcium levels were similar at the start of the study; however pigs on the high vitamin D_3 treatment had higher ending serum calcium concentrations than pigs fed the control or moderate vitamin D_3 diets. Pig weight off test and average daily gain were reduced for pigs on the high vitamin D_3 diets compared to pigs fed the control or moderate level of vitamin D_3 diets. Average daily feed intake was also decreased in pigs fed high levels of dietary vitamin D_3 compared to the other diets.

Carcass and meat quality results are presented in Table 3 and 4 respectively. Pigs fed a high level of vitamin D_3 were lighter at slaughter and had a reduced carcass weight but an increased dressing percent compared to the other diets. Drip loss was reduced by two percentage in pigs fed the high level of vitamin D_3 compared to pigs on the moderate level or the control diet. There was a decrease in Hunter color scores with increasing dietary vitamin D_3 level suggesting that pigs fed high levels of vitamin D_3 have darker longissimus color.

The effects of vitamin D_3 levels and aging are presented in Table 5. There was no interaction between vitamin D_3 level and aging time, suggesting that the effects of those two treatments are independent and additive. There was no difference in Warner-Bratzler shear force scores between pigs fed different dietary levels of vitamin D_3; however pigs fed a high level of vitamin D_3 had a higher percentage weight loss during cooking when compared to pigs fed a control diet or moderate level of vitamin D_3. In addition, there was no effect of aging time on eating quality characteristics when samples were aged for seven or fourteen days.

Feeding high levels of vitamin D_3 during the last ten days of the finishing period has been shown to increase tenderness in beef (Whittle, 1997). In addition, aging of vacuum-packaged longissimus sections from steers fed high levels of vitamin D_3 resulted in decreased Warner-Braztler shear force scores (Whittle, 1997). This increase in tenderness is thought to be the result of increased activity in the calpain family of enzymes, in particular m-calpain enzymes. However, our results indicate that elevating the level of dietary vitamin D_3 and serum calcium concentrations did not increase the tenderness attributes of the longissmus muscle in pork. In addition, juiciness and off-flavor were not affected by aging treatment or level of dietary vitamin D_3. The lack of improvement in eating quality in pigs fed vitamin D_3 may be genotype specific.

Indicating that the use of the Duroc or Duroc crosses which had good meat quality may have limited the tenderness response from vitamin D_3. However feeding vitamin D_3 did improve objective color scores. In addition, drip loss was reduced in the presence of elevated serum calcium levels as a result feeding high levels of vitamin D_3.

Conclusions

1. Feeding high levels of vitamin D_3 during the final ten days of the finishing period reduced feed intake levels and growth performance.

2. Supplementing diets with high levels of vitamin D_3 in swine resulted in reduced drip loss and improved color and firmness scores in loin chops.

3. Vitamin D_3 supplementation did not improve tenderness and juiciness of loin chops.

4. Further research is necessary to determine the appropriate feeding level and timeof for vitamin D_3 to improve meat quality in pigs.

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Table 1. Percentage composition of diets [a].

	Dietary vitamin D_3 level		
Ingredient	*Low*	*Moderate*	*High*
Corn	78.38	77.84	76.43
Soybean meal(48 % CP)	19.00	19.00	19.00
Dicalcium phosphate	1.250	1.000	1.250
Limestone	.775	1.000	.775
Trace mineral salt[b]	.350	.350	.350
Vitamin premix[c]	.100	.100	.100
Copper sulfate	.100	.100	.100
Tylan (2%)	.050	.50	.50
Vitamin D3 (9 mil IU/lb)	0.00	.556	1.944
Calculated composition			
Crude protein,%	15.78	15.74	15.62
Calcium,%	.631	.661	.630
Phosphorus, Total,%	.574	.526	.569
Phosphorus, Avail,%	.295	.248	.294
Swine ME, kcal/kg	3323	3305	3257
Lysine,%	.781	.780	.776
Vitamin D (1000 IU)	.331	50.040	176.000

[a] As-fed basis

[b] Each kilogram of mix contained the following: Se, 85.7 mg; I, 100 mg; Cu, 2.3 g; Mn, 5.7 g; Fe, 25.7; Zn, 28.6 g; NaCl, 855 g.

[c] Each kilogram of mix contained the following: vitamin A, 3,000,000 IU; vitamin D_3, 330,000 IU; vitamin E, 44,000; vitamin K, 2.2g; vitamin B_{12}, 17.9 mg; riboflavin, 4.4 mg; d-pantothenic acid, 12.1g; ; niacin, 16.5 g; choline chloride, 165 g; and roughage products to 1 kg.

Table 2. Least square means for the effects of dietary levels of vitamin D_3 on growth performance traits.

Variable	*Dietary vitamin D_3 level*			*Ave SE*	*Sig.**
	~~*Level*~~ *Low*	*Moderate*	*High*		
Serum Calcium, μ/ml					
At start of feeding period	9.50	9.46	9.62	.108	NS
At end of feeding period	9.43[a]	10.27[b]	13.78[c]	.263	***
Growth performance					
Weight on test, kg	104.5	103.8	105.6	1.43	NS
Weight off test, kg	113.5[a]	111.5[a]	106.7[b]	1.82	*
Average daily gain, kg/d	.78[a]	.67[a]	.07[b]	.164	*
Average daily feed intake, kg/d	3.82[a]	3.63[b]	2.90[c]	.005	***

[abc](kg/d)Means in the same row with differing subscripts differ.

* NS, *, **, *** = not significant, P<.05, P<.01, P<.001 respectively.

Table 3. Least squares means for the effects of dietary levels of vitamin D_3 on carcass quality measurements.

	Dietary vitamin D_3 level				
~~*Level*~~					
Variable	*Low*	*Moderate*	*High*	*Ave SE*	*Sig.**
Final live weight, kg	112.83[a]	109.41[a]	102.78[b]	1.58	**
Hot carcass weight, kg	84.56[a]	82.00[a]	79.19[b]	1.39	**
Cold carcass weight, kg	82.61[a]	80.11[a]	75.73[b]	1.34	**
Dressing percent, %	74.95[a]	74.96[a]	77.09[b]	.556	*
Carcass length, cm	81.98	81.13	80.45	.507	NS
Loin eye area, $cm^{2,}$	34.45	35.01	34.99	.957	NS
Loin eye depth, cm	5.40	5.50	5.23	.102	NS
Fat depth 1^{st} rib, cm	4.62	4.75	4.17	.212	NS
Fat depth 10^{th} rib, cm	2.59	2.61	2.23	.203	NS
Fat depth last rib, cm	2.54	2.48	2.43	.183	NS

[abc] Means in the same row with differing subscripts differ.

* NS, *, **, *** = not significant, P<.05, P<.01, P<.001 respectively.

Table 4. Least square means for the effects of dietary levels of vitamin D_3 on meat quality measurements.

	Dietary Vitamin D_3 level				
Variable	*Low*	*Moderate*	*High*	*Ave SE*	*Sig.* *
Color[a]	2.08^{b}	2.72^{a}	3.08^{a}	.198	**
Intramuscular Fat[a]	2.08	2.17	2.67	.255	NS
Firmness[a]	2.25^{a}	2.62^{ab}	2.92^{b}	.181	*
pH	5.50	5.53	5.47	.039	NS
Drip loss, %	4.39^{a}	3.21^{ab}	2.04^{b}	.593	*
Hunter L*	54.58^{a}	52.49^{ab}	51.20^{b}	1.02	NS
Hunter a*	6.33	6.43	6.54	.205	NS
Hunter b*	16.69^{b}	15.99^{a}	15.64^{a}	.209	**

[a] Subjective score from 1= extremely pale, soft, and devoid of marbling to 5 = extremely dark, firm, and moderately abundant or greater marbling.

[abc] Means in the same row with differing subscripts differ.

* NS, *, **, *** = not significant, P<.05, P<.01, P<.001 respectively.

Table 5. Least square means for the effect of dietary vitamin D_3 levels on aging treatments.

	Dietary vitamin D_3 level				*Aging time*		
Variable	*Low*	*Moderate*	*High*	*Ave SE*	*7 Days*	*14 Days*	*Ave SE*
Juiciness[a]	9.08	9.22	9.16	.22	9.25	9.06	.179
Tenderness[a]	8.69	9.11	8.54	.22	8.83	8.74	.180
Off-flavor[a]	14.80	14.83	14.75	.043	14.80	14.78	.035

[a] Subjective score from 0 = extremely dry, tough, and intense off-flavor to 15 = extremely moist, tender, and no off-flavor.
[abc] Means in the same row with differing subscripts differ.
* NS, *, **, *** = not significant, P<.05, P<.01, P<.001 respectively.

PERFORMANCE OF ONE-QUARTER CHINESE (MEISHAN) AND THREE-BREED CONVENTIONAL CROSSES FOR SOW PRODUCTIVITY AND GROWTH AND CARCASS CHARACTERISTICS OF THE PROGENY.

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Introduction

For many years, crossbreeding programs based on Western pig breeds have been used to improve sow productivity through maximizing maternal heterosis (Kuhlers et al., 1988; McLaren et al., 1987). In the last decade, in both the U.S. and Europe the Machine, a highly prolific Chinese pig breed, has been evaluated for use in commercial crosses (Bidanel et al., 1991; Mercer and Hoste, 1994; Lee and Haley, 1995; Sinclair et al., 1998; Young, 1998). Levels of heterosis between Machine and Western breeds are higher than crosses between Western breeds (Mercer and Hoste, 1994). Therefore, there is an interest in developing crossbreeding programs that utilize the Machine and Western breeds in order to exploit this heterosis and the prolificacy of the Machine.

Machine F1 crossbred females have been found to improve reproductive and litter productivity traits compared to F1 crosses of Western breeds (Lee and Haley, 1995; Young, 1995a). However, growth performance and carcass composition of the progeny is significantly poorer than that of Western breeds. It is therefore of no economic benefit to utilize the Machine F1 in typical commercial pig production.

However, female lines with one-quarter Machine have been developed and the objective of this study was to compare reproductive, litter, and progeny growth and carcass performance of two 1/4 Machine sow lines and a three-breed conventional cross for traits of commercial importance.

Experimental Approach

The study was conducted at the Moorman Swine Research Farm at the University of Illinois. Three dam lines were produced for this study; namely Landrace x (Machine x Yorkshire) (LMeY), Duroc x (Machine x Yorkshire) (DMeY), and Landrace x (Duroc x Yorkshire) (LDY). In addition, a performance test was conducted to evaluate the progeny of the three dam lines mated to terminal Hampshire boars.

At the start of the study, 45 (LMeY), 41 (DMeY), and 50 (LDY) gilts were selected on the basis of dam and sire, with no greater than four gilts being kept from any one dam and at least eight sires being represented in each genotype. Sows were kept for five parities producing a total of 142, 153, 157 litter records for genotypes LMeY, DMeY, and LDY, respectively.

A total of 170 progeny with 60 pigs from both LMeY and DMeY lines, and 50 pigs from the LDY line were evaluate for growth performance and carcass characteristics. Progeny originated from five Hampshire sires and at least 15 dams from each genotype were represented.
Sow and Litter Management and Housing. Gilts selected for this experiment were group-housed in a breeding facility at approximately 220 days of age with fence-line exposure to boars. All matings were to Hampshire boars utilizing artificial insemination. Gilts were pen gestated with eight animals per pen. In subsequent parities sows were housed in gestation stalls. The gestation facilities were environmentally controlled and mechanically ventilated. During breeding and pregnancy sows were subject to standard commercial management practices and were fed approximately 2.2 kg of a corn-soybean meal diet (formulated to contain 12 % CP, 9g/kg of lysine, and 3300 kcal/kg ME) daily.

Sows were weighed and moved to the farrowing accommodation at day 108 of gestation and remained in individual farrowing crates in controlled environment rooms for the entire farrowing and lactation period. Pre-farrowing sows were fed approximately 3.0 kg of a corn-soybean lactation diet (formulated to contain 13% CP, 27g/kg of lysine, and 3400 kcal/kg ME) daily. From immediately post-farrowing, they were fed twice daily to appetite. Within 24 h of farrowing, number of live born, stillborn and number of mummified piglets and piglet weights were recorded, males were castrated, needle teeth were clipped, ears were notched for identification, and piglets were given 1 ml of iron dextran and 1ml penicillin as preventative medication for scours. Cross-fostering occurred when sows farrowed greater than 12 piglets. In those instances, extra pigs were transferred to other sows with smaller litters of similar age and piglet size to try to equalize litters to 10 pigs. Pigs were individually weighed at 21 days and cross fostered pigs were weighed with the recipient sow's litter. Piglets were weaned at 28 days of age into a conventional nursery. Sows were individually weighed and placed in group-housing for re-breeding, and the weaning to farrowing interval was recorded. Death, lameness, illness, and failure to return to estrus resulted in the culling of both gilts and sows. The number of culls were recorded throughout the study and the culling rate was determined by dividing the number of sows culled prior to the fifth parity by the total number of gilts available for breeding at the start of the study.

Grow-Finish Management and Housing. Animals were allotted on the basis of weight to like-genotype, like-sex pens of five pigs at an average start weight of 40 kg and taken off test at an average live-weight of 110 kg. During the study, pigs were housed in two different environmentally controlled finishing houses with partially slatted flooring and a pen space allocation of 1.2 m^2/pig. Pigs were given ad libitum access to feed from a two hole feeder. A two-phase dietary program was used with diets being based on corn and soybean meal and formulated to meet or exceed NRC (1988) nutrient requirements. Pigs were given ad libitum access to water via a nipple waterer in each pen. Live weight and feed consumption were recorded at 14-d intervals. In addition, pigs were ultrasonically scanned to measure backfat and loin depth anterior to the last rib, 5 cm off of the midline at the start and at 28-d intervals throughout the trial.

Statistical analysis. All results were analyzed using the GLM procedure of SAS (1990). The model used for reproductive and litter productivity traits (traits of the dam) included the effects of genotype, farrowing season, litter sire, parity of dam, genotype x season interaction, genotype

x parity interaction, and dam nested in genotype. Dam nested within genotype was used as the error term to test genotype effects.

For progeny performance test data, the pen was used as the experimental unit for feed intake, growth and feed efficiency, and the individual animal was used as the experimental unit for carcass data. Analysis of growth performance and feed efficiency was performed using a model that included the effects of genotype, sex, finishing house, genotype x sex interaction, finishing house x sex interaction, finishing house x genotype interaction.

Results and Discussion

Reproductive performance is summarized in Table 1. Gestation length was similar for all three genotypes. However, LMeY had a shorter farrowing interval than LDY, with DMeY being similar and intermediate to the other two lines. This suggest that LMeY either had a shorter return to estrus interval from weaning and/or fewer returns to estrus after breeding than LDY. European research (Mercer and Hoste, 1994), reported that sows containing Machine had shorter return to estrus than conventional white-cross sows. In contrast, Young (1998a) reported no significant differences for weaning to first estrus interval for a (1/4 Machine, 3/4 White) composite line compared to a (1/4 Duroc, 3/4 White) composite line. However, Young (1995a) comparing (½ Machine, ½ White) composite verses (½ Duroc, ½ White) composite females, found that the Machine line had a significantly shorter weaning to estrus interval. The shorter interval to estrus exhibited by the LMeY line would result in fewer non-productive days, increasing the efficiency of production compared to the DMeY and LDY lines. Additionally, the culling rate or the percentage of sows removed before parity five was lower for DMeY than for LDY, with LMeY being intermediate. Female replacement rate has been shown to have a substantial impact on the efficiency of a swine crossbreeding system (McLaren et al., 1987). Therefore, the sow genotypes containing Meishan in this study would have a significant advantage over the conventional three-breed cross in this respect.

Farrowing performance was generally similar between the three genotypes. Total pigs born and number of live born pigs did not differ between genotypes. U.S. studies (Young 1998), found no significant differences between (1/4 Meishan, 3/4 White) composite and (1/4 Duroc, 3/4 White) composite lines for number born alive. However, Mercer and Hoste (1994) suggested that there is a significant advantage in number born alive in a synthetic sow line containing 1/4 Meishan relative to a conventional White sow line. Other studies have also indicated an advantage in number born alive for the Meishan crosses over Western breed crosses (Bidanel et al., 1989; Haley et al., 1995). Individual pig birth weights in the current study were similar for all genotypes, however, LMeY had significantly higher live born litter birth weights than either DMeY or LDY. Young (1998) also reported no difference in piglet birth weights between (1/4 Meishan, 3/4 White) composite and (1/4 Duroc, 3/4 White) composite genotypes. In contrast, most research has found sows containing 1/4 Meishan to produce lighter piglets at birth (Mercer and Hoste, 1994; Sinclair et. al, 1998; Van Der Steen and De Groot, 1992).

Pre-weaning mortality was significantly lower for the Meishan lines than for the LDY. Therefore, numbers weaned were higher for LMeY than LDY with DMeY being intermediate for

this trait. This may have resulted from behavioral differences between the genotypes. There is evidence (Sinclair et al., 1998) that ½ Meiahan sows spend less time standing with fewer posture changes during lactation when compared to sows of a synthetic white line genotype. Furthermore, van der Steen and de Groot (1992) found that Meishan sows possess good maternal characteristics with a resulting lower piglet mortality and even heavier piglets at weaning, and Haley and Lee (1994) have shown that at a constant birth weight Meishan cross piglets have an advantage in survivability.

The LMeY sows were significantly lighter pre-farrowing than the other two dam lines. In addition, the two Meishan genotypes lost significantly more body weight during lactation than LDY. LMeY had a higher percentage of body weight loss than both DMeY and LDY, losing 21.7 percent of pre-farrowing body weight compared to 18.5 and 14.9 for DMeY and LDY, respectively. These results are similar to those of Sinclair et al. (1996) who showed that ½ Meishan sows had a lower live weight pre-partum and higher total live weight loss post-partum compared to White line sows. Additionally, the sows of Meishan genotype also suffered a higher backfat loss over the tenth rib during lactation and had a higher litter growth rate than the White line sows (Sinclair et al., 1996).

Growth performance and carcass measurements for genotype. Performance and carcass composition data for the progeny from the three crossbred lines are presented in Table 2. Average daily gain, daily feed intake, and gain to feed ratio did not differ between the three genotypes. Young (1998b) also reported no differences in the growth performance and feed efficiency between (1/8 Meishan, 7/8 White) and (1/8 Duroc, 7/8 White) pigs. Both European and U.S. studies, however, have reported that purebred Meishan and Meishan crossbred pigs containing at least 1/4 Meishan have reduced growth rates with poorer feed efficiency than Western breeds of pigs (Bidanel et al., 1993; Haley et al., 1992; Mercer and Hoste, 1994; White et al., 1994; Young, 1995b). While Meishans generally exhibit lower growth performance traits than Western breeds, 1/8 Meishan pigs appear to have similar growth performance compared to other commercial Western breed crosses.

Ultrasound fat and loin eye measurements were corrected to an average end of test live weight of 114 kg. There were no differences for either backfat or loin depth at the last rib between genotypes. These findings are in agreement with data reported by Young (1998b) where no differences were found between pigs with 1/8 Meishan compared to conventional genotypes for level of backfat at slaughter. However, Young (1998b) found that (1/8 Duroc, and 7/8 White) composite line pigs did have higher yield of lean cuts than those of the 1/8 Meishan line.

Conclusions

1. The LMeY dam line had improved reproductive performance compared to the conventional three-breed cross.

2. The lower overall body weight in addition to the lower culling rate of the LMeY female line may correspond to a reduction in the cost of maintaining this genotype in commercial swine operations.

3. Growth performance and carcass composition to typical U.S. slaughter weights was not significantly different for the progeny of 1/4 Meishan sows compared to those of a conventional three-breed cross in this study.

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Table 1. Least square means for reproductive and litter productivity traits in the crossbred female lines.

Items	*Dam Genotype*[d]			*Ave SE*	*Sig*
	LMeY	DMeY	LDY		
Number of Sows	45	41	50	-	-
Reproductive Performance					
Gestation Length, day	114.5	114.3	115.0	0.43	NS
Farrowing Interval, day	143.5[a]	145.0[ab]	149.1[b]	3.07	*
Culling Rate, %	53.0	41.0	60.0	-	-
Farrowing Performance					
Total Number Born	11.82	11.35	11.25	0.762	NS
Number Born Alive	10.67	10.19	9.92	0.640	NS
Number Still Born	0.99	0.98	1.22	0.294	NS
Number Mummified	0.17	0.19	0.12	0.158	NS
Average Piglet Weight, kg	1.88	1.89	1.83	0.010	NS
Total Litter Weight, kg	20.28[a]	18.45[b]	17.54[b]	1.085	**
Weaning Performance					
Pigs Weaned/Litter	10.44[a]	9.80[ab]	9.28[b]	0.620	*
Pre-weaning Mortality, %	3.1[a]	4.2[a]	6.4[b]	1.59	**
Total Litter 21 d Weight, kg	52.95	51.25	53.29	2.992	NS
Sow Weights					
Pre-Farrowing, kg	235.16[a]	247.79[b]	247.06[b]	7.020	*
Post-weaning, kg	190.05[a]	206.72[b]	213.35[b]	6.916	**
Lactation Weight Loss, kg	51.30[a]	45.38[b]	37.20[c]	4.198	*
Lactation Weight Loss, %	21.7[a]	18.5[b]	14.9[c]	0.016	**

1 kg = 2.2046 lbs

[a, b, c] Means within row with differing superscripts differ (P<.05).

NS, *,**, *** = not significant, P<.05, P<.01, P<.001 respectively.

[d] LMeY = Landrace x (Machine x Yorkshire), DMeY = Duroc x (Machine x Yorkshire), and LMY = Landrace x (Duroc x Yorkshire)

Table 2. Least square means for genotype and sex for progeny growth performance traits.

	Dam Genotype					*Sex*			
Variable	*LMeY*	*DMeY*	*LDY*	*Avg SE*	*P value*	*Barrow*	*Gilt*	*Avg SE*	*P value*
Growth performance traits									
Start of test weight, kg.	40.0	40.6	40.6	0.92	NS	40.2	40.5	0.75	**
End of test weight, kg.	107.8	107.6	109.1	1.51	NS	109.5	106.8	1.23	NS
Days on test	82	80	82	1.5	NS	80	83	1.2	NS
Average daily gain, g									
40 - 80 kg	816	815	857	24.5	NS	860	799	19.8	**
80 - 110 kg	921	960	985	34.0	NS	995	916	27.5	**
40 - 110 kg	868	888	921	18.3	NS	927	858	14.8	***
Gain:feed									
40 - 80 kg	0.37	0.36	0.36	0.014	NS	0.36	0.36	0.012	NS
80 - 110 kg	0.29	0.30	0.31	0.010	NS	0.29	0.30	0.008	NS
40 - 110 kg	0.33	0.33	0.34	0.010	NS	0.33	0.33	0.008	NS
Average daily feed intake, g									
40 - 80 kg	2315	2346	2394	68.1	NS	2470	2234	55.0	***
80 - 110 kg	3276	3285	3178	85.9	NS	3433	3060	69.4	***
40 - 110 kg	2795	2816	2786	57.7	NS	2951	2647	46.6	***
Carcass traits[a], last rib, mm									
Fat depth	20.74	19.01	18.72	0.852	NS	20.94	18.04	0.442	***
Loin eye depth	52.16	52.63	54.30	0.764	NS	51.95	54.09	0.595	***

1 kg = 2.2046 lbs; 1 mm = 0.039 in.

[a] All measurements corrected to a live weight of 114.37 kg using covariate analysis.

NS, **, *** = not significant, P<.05, P<.01, respectively.

EFFECTS OF LONGISSIMUS GLYCOLYTIC POTENTIAL LEVELS ON GROWTH PERFORMANCE, CARCASS, AND MEAT QUALITY CHARACTERISTICS IN A POPULATION OF HYBRID PIGS WITH HAMPSHIRE ANCESTRY

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Introduction

The Napole gene has been shown to increase muscle glycogen levels and glycolytic potential and result in increased lactate production postmortem and produces acidic meat which has a lower than normal pH (Monin and Sellier, 1985). This acidic meat has reduced processing yield and increased purge loss, both of which decrease profits for the packer. However, Sutton (1997) showed that pork with low ultimate pH had improved palatability traits when measured at 10 d postmortem. In sheep, Watanabe et al. (1996) reported that higher ultimate pH was unfavorably related to shear force at 2 d post mortem but not after a 5 d period of ageing. Other authors have also reported increased tenderness with increased ageing time in pork (Weakley et al., 1986; Tornberg et al., 1994; Taylor et al., 1995), while the beef industry has utilized ageing to improve beef tenderness for many years (Smith et al., 1978). There is, however, no data on the interaction between ageing time and glycolytic potential for eating quality and this study was carried out to investigate this interaction. In addition, the effects of glycolytic potential on growth, carcass and meat quality traits and a rapid method of classification using free glucose concentrations were also studied.

Materials and Methods

Trial design and performance data. This study was conducted at the Swine Research Center at the University of Illinois and used progeny of line 355 sires and Camborough 22 dams (Pig Improvement Company, USA). The sires had Hampshire ancestry and previous research had indicated that they were likely to be heterozygous for the Napole gene.

Biopsy sampling and glycolytic potential determination. Prior to the start of the performance test, animals were housed in conventional accommodation and subjected to standard commercial management. A total of 150 animals had a longissimus sample removed using spring loaded biopsy equipment (Biotech PPB-U, Nitra, Slovakia). The depth was set to penetrate 5.8 cm and samples were taken from the longissimus at the level of the last rib, 5 cm from the midline, from the right side of the animal. Samples were immediately frozen in liquid nitrogen and subsequently freeze dried. Glycogen, glucose, glucose-6-phosphate, and lactate concentrations were determined on the freeze dried biopsy sample by enzymatic assay, using standard procedures.

Glycolytic potential values were calculated using the formula described by Monin and Sellier, (1985) as follows:

Glycolytic potential = 2 ([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate].

A frequency distribution of glycolytic potential was created and animals were classified as having low, moderate, and high glycolytic potential on the basis of this distribution. The low group had glycolytic potential between 154 and 177 μmol / g, the moderate group had values between 184 and 217 μmol / g and the high group had glycolytic potentials between 220 and 301 μmol / g.

Trial design and performance test. A total of 72 pigs, with equal numbers of barrows and gilts, were put on test at a live weight of approximately 80 kg and were taken to a weight of 125 kg. Pigs were allotted on the basis of weight to like-glycolytic potential classification, like-sex groups of four animals giving six groups per glycolytic potential classification. During the study, pigs were housed in a controlled environment finishing house which had partially slatted floors and a space allowance of 1.2 m^2 per pig. Animals were given ad libitum access to a finisher diet based on corn and soybean meal from a two-hole feeder, and had unlimited access to water. The diet was formulated to supply 15.8 % protein and 3329 kcal/kg ME. Pigs and feeders were weighed weekly and feed additions were recorded. Pens of pigs were taken off test and sent for slaughter when their average weight was within 3 kg of the target weight of 125 kg. On the afternoon prior to slaughter, animals were weighed before loading, and transported to a slaughter plant which was located approximately 170 miles from the farm. On arrival at the slaughter facility, the animals were held in lairage, without food but with access to water, for approximately 16 h before slaughter. Pigs were mixed in one group during transport and in the lairage. Animals were slaughtered using standard commercial procedures.

Carcass evaluation. At 24-h postmortem, cold carcass weights were recorded, and carcass measurements were obtained on the right side of each carcass. These measurements included: carcass length (measured from the cranial tip of the aitch bone to the cranial edge of the first rib adjacent to the thoracic vertebra), midline fat measurements (opposite the first rib, last rib, and last lumbar vertebra), and tenth rib measurements which included loin eye depth and area, and fat depth over the loin eye, 5 cm from the midline.

Meat quality measurements. The longissimus muscle from the right side of each pig was transported to the Meat Science Laboratory at the University of Illinois for further analysis. Ultimate longissimus pH was determined by the removal of a muscle sample (approximately 3 g) from the longissimus at the level of the l0th rib with a no.3, 8 mm cork bore and homogenized in 10 ml of iodacetate. The pH of the sample was determined using an Orion model 720A pH meter fitted with a Ross Sure Flow 81-72 electrode (Orion Research, Boston, MA). Subjective longissimus color, firmness, and marbling scores were taken using the procedures described by NPPC (1991). Three loin chops (2.5 cm thick) were obtained from the longissimus. One chop was weighed, placed in a Whirl-pak bag, and suspended in a 4°C cooler for 48 h then re-weighed and drip loss recorded. One chop was trimmed of epimysium and external fat, homogenized, then placed in a Whirl-pak bag and frozen (-20°C) for subsequent chemical analysis. The remaining chop was used for glycolytic potential determination using the procedures previously described for the biopsy sample. The remainder of the longissimus was divided into three

sections which were weighed prior to being vacuum packaged and later weighed after thawing to determine purge loss. Sections were randomly assigned to one of three ageing treatments (2, 9 or 21 d) Hunter color (L*, a*, and b*) was measured on the cut surface of the three sections using a Hunter LabScan Spectrocolormeter, Model XE (Hunter Associates Laboratory, Inc., Reston, VA). Samples were placed in cooler (4°C) and allowed to age for the designated number of days at which stage they were frozen (-20°C) for subsequent sensory evaluation and Warner-Bratzler shear force determination.

Chemical analysis. Fat and water contents were determined on the homogenized longissimus sample using the procedures described by Novakofski et al. (1989). All samples were oven dried (110°C for 48 h) and fat was extracted using a mixture of warm chloroform and methanol (4:1).
Shear force and sensory evaluation. Chops for Warner-Bratzler shear force were thawed for 24 h at 4°C and cooked on Farberware open hearth grills (Model 1 SSN, Walter Kidde, Bronx, NY) to an internal temperature of 70°C. Temperatures were monitored using copper Constantan thermocouples and a recording thermometer (Campbell Scientific, Logan, UT). Chops were weighed before and after cooking to determine cooking loss. Chops were cooled to 25°C and six 1.3 cm diameter cores were removed parallel to the muscle fibers. Shearing was accomplished with an Instron Model 1122 Universal Testing Machine (Instron, Canton, MA) fitted with a Warner-Bratzler shear attachment. The full scale load was set at 10 kg and the chart drive and crosshead speed was 200 mm/min. Chops for sensory evaluation were prepared and cooked using the same procedures as for shear force. Twelve panelists consisting of faculty and graduate students from the Meat Science Laboratory, who have been trained according to the procedure for sensory evaluation described by the American Meat Science Association (1978), were used. A taste panel consisting of 6 trained panelists evaluated tenderness, juiciness, and off-flavor intensity using a 15 cm structured line scale with anchors and a mid-point (0 cm extremely dry, tough, and intense off flavor; and 15 cm = extremely moist, tender, and no off flavor). Water was provided to panelists to cleanse the palate between samples.

Free glucose determination. A Pipetteman was used to remove a 5 ul sample of the purge loss from the post-mortem longissimus sample that had been used for glycolytic potential determination. The sample was analyzed using a devise developed for checking blood glucose levels in diabetes patients (Accu-Chek Instant Blood Glucose Monitoring System, Boehringer Mannheim Corporation, Indianapolis, IN). This process involved the addition of the 5 ul sample to a test strip and feeding the strip into the glucose analyzer. The process from this point takes approximately 12 seconds, and the glucose concentration (mg/dl) is provided.

Results and Discussion

Glycolytic potential levels determined from live-animal biopsy samples were different among the three phenotypes, while values from postmortem samples and the free glucose levels were different between high and low, and high vs moderate groups, but not between low and moderate groups (Table 1). Least square means for growth performance and carcass traits are presented in Table 2 and 3, respectively. There were no differences between glycolytic potential classifications for those traits. However, there was a trend for high glycolytic potential pigs to

have higher average daily gain, less backfat thickness and larger loin eye area which is similar to results reported by LeRoy et al. (1996) and Enfalt et al. (1997).
Evaluation of meat quality traits showed several differences among glycolytic potential classifications (Table 4). Longissimus ultimate pH was significantly lower for animals with high compared to low glycolytic potential levels with the moderate group being intermediate in this respect. Several authors have reported similar results for ultimate pH for animals with high compared to low glycolytic potential (Monin and Sellier, 1985; Enfalt et al., 1994; LeRoy et al., 1996; Lundstrom et al., 1996; Sutton, 1997). Pigs with high and moderate glycolytic potential had higher Hunter L* and b* values, and lower subjective color scores compared to those classified as low. Drip loss was greater for the high compared to the moderate and low groups (Table 4). Decreased water holding capacity and paler fresh pork color has been reported by several authors (Enfalt et al., 1994; Lundstrom et al., 1996; Sutton, 1997). Longissimus protein content was different between the glycolytic potential classes, with the low and moderate groups having higher values compared to pigs classified as high (Table 4). Sensory evaluation by a trained taste panel showed significant tenderness and juiciness advantages for the high glycolytic potential group compared to the low and moderate groups (Table 5). LeRoy et al. (1996) reported both tenderness and juiciness advantages of 12.7 and 6.8 percent, respectively, for pigs with high compared to low glycolytic potential.

Purge loss was highest for the 9-d ageing treatment, lowest for 21-d ageing and intermediate for the 2-d ageing treatment (Table 5). However, cooking loss was not different for the three ageing periods. Increasing ageing time from 2 to 21 days decreased shear force and increased tenderness scores. Tenderness scores from loins aged 9 days were intermediate yet significantly different from both 2 and 21 day ageing treatments. Ellis et al. (1998) reported similar results with .17 kg decrease in shear force and .4 unit increase in tenderness and juiciness scores by increasing ageing time from 2 to 16 days. The continued increase of tenderness to day 21 in this study is in contrast to the results reported by Weakley et al. (1986), who saw limited tenderness improvements in longissimus aged for more than 7 days. Tornberg et al. (1994) and Taylor et al. (1995) have reported tenderness improvements in longissimus aged from 4 to 12 days, however limited data is available investigating ageing treatments greater than 21 days. There were significant interactions between glycolytic potential and ageing time for juiciness and off-flavor scores (Table 5). At 2-d postmortem juiciness scores were significantly lower for low and moderate classified animals compared to animals with high glycolytic potential. In addition, juiciness scores for the moderate group were higher than for the low group after 9- and 21-d ageing, but not at 2-d post mortem. For off-flavor, there was no difference between the glycolytic potential classifications after ageing periods of 2-d and 9-d (Table 5); however, in samples aged for 21-d, the low group had reduced scores compared to the other two group. This indicates that there were more off-flavors in the low glycolytic potential group after 21-d ageing, although the difference was relatively small. Lundstrom et al. (1996) reported an increased acid flavor for pigs who carry the RN^- allele and have high glycolytic potential levels and higher than normal levels of lactic acid present in the muscle post mortem.

Implications

The results of this study highlight the effects of both glycolytic potential levels within the muscle and ageing time on meat quality traits. Higher glycolytic potential levels may cause increased economic loss for the packer and poorer visual appearance due to decreased water holding capacity and paler color in the fresh product. However, consumer satisfaction could be positively affected due to increased tenderness and juiciness, which was also increased through the ageing process. The ability to identify pigs with different glycolytic potential levels and in combination with varying post-mortem ageing times could lead to specific marketing of the fresh product.

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Table 1. Least square means for glycolytic potential classifications for sampling methods.

Traits	*Glycolytic potential classification*			*Ave SE*
	Low	*Moderate*	*High*	
Glycolytic potential:				
Biopsy sampling (umol/g)	154.3 [a]	196.8 [b]	253.4 [c]	4.08
Post mortem sampling (umol/g)	134.5 [a]	149.1 [a]	170.9 [b]	6.16
Free Glucose (mg/dl)	78.9 [a]	109.9 [a]	167.6 [b]	15.36

[a.b.c] Means within a row with different superscripts are significantly different ($P < .05$).

Table 2. Least square means for glycolytic potential classifications for growth performance.

Traits	*Glycolytic potential classifications*			*Ave SE*
	Low	*Moderate*	*High*	
Weight at start of test, kg	85.9	85.2	85.6	1.19
Weight at end of test, kg	121.0	120.3	120.0	1.67
Days on test	42.0	38.5	39.7	2.33
Average daily feed intake, kg	3.05	3.07	2.95	.085
Average daily gain, g	835	930	871	46.7
Gain: feed, kg	.273	.290	.296	.0146

Table 3. Least squares means for glycolytic potential classification for carcass measurements

	Glycolytic potential classification			
Traits	*Low*	*Moderate*	*High*	*Ave SE*
Final live weight, kg	127.4	127.5	126.1	1.33
Cold carcass wt, kg	91.1	91.7	90.4	1.03
Dressing percent (cold)	71.5	72.0	71.7	0.37
Carcass length, cm	85.2	85.3	84.2	.44
Midline fat measurements, cm				
First rib	4.05	4.31	3.96	.127
Last rib	2.38	2.45	2.27	.074
Last lumbar	1.98	2.03	1.95	.086
Tenth rib measurements, cm				
Fat depth (½)	2.21	2.29	2.02	.094
Loin eye depth (½)	6.17	6.17	6.32	.139
Loin eye area, cm^2	40.4	41.4	42.0	1.31

Table 4. Least square means for meat quality characteristics measured on the longissimus.

	Glycolytic potential classifications			
Traits	*Low*	*Moderate*	*High*	*Ave SE*
pH 24 hrs.	5.62 [a]	5.54 [ab]	5.47 [b]	.051
Color [d]	2.58 [b]	2.22 [a]	2.38 [a]	.131
Firmness [d]	2.03	1.88	1.86	.147
Marbling [d]	2.47	2.36	2.55	.152
Hunter color values:				
L*	51.6 [b]	54.1 [a]	54.1 [a]	.87
a*	5.9	5.8	6.0	.17
b*	16.0 [b]	16.5 [a]	16.5 [a]	.18
Drip loss, %	5.04 [a]	5.60 [a]	7.09 [b]	.520
Chemical Analysis, % :				
Protein	24.1 [a]	23.8 [a]	23.3 [b]	.19
Moisture	73.6	73.6	74.1	.18
Fat	2.22	2.08	1.99	.17

[a b,c] Means within a row with different superscripts are significantly different ($P < .05$).

Table 5. Glycolytic potential and ageing time least square means for eating quality characteristics.

	Glycolytic potential Classification				*Ageing treatments (days)*			
Trait	*Low*	*Mod*	*High*	*Ave SE*	*2*	*9*	*21*	*Ave SE*
Purge loss, %	5.86 [a]	6.56 [ab]	7.50 [b]	.390	6.82 [ab]	7.26 [b]	5.85 [a]	.390
Cooking loss, %	19.84	21.27	21.43	.602	21.17	20.53	20.83	.602
Shear force, kg	2.66 [a]	2.73 [a]	2.49 [b]	.051	2.73 [a]	2.59 [ab]	2.55 [b]	.051
Taste panel evaluation:								
Tenderness	9.04 [a]	9.79 [b]	10.44 [c]	.121	9.17 [a]	9.78 [b]	10.33 [c]	.121
Juiciness								
2 day	7.94 [a]	7.87 [a]	8.60 [b]	.212				
9 day	8.09 [b]	9.00 [a]	8.73 [a]					
21 day	8.34 [b]	9.28 [a]	8.98 [a]					
Off-flavor								
2 day	14.49	14.46	14.38	.116				
9 day	14.57	14.27	14.53					
21 day	14.11 [b]	14.44 [a]	14.63 [a]					

[a,b,c] Means within a row with different superscripts are significantly different ($P < .05$).
[d] Significant interactions were included in table.

NAPOLE (RN) GENE FREQUENCY WITHIN THE UNITED STATES HAMPSHIRE POPULATION

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Introduction

The Napole gene has been shown to have detrimental effects on pig meat quality (Enfalt et al., 1994; LeRoy et al., 1996; Lundstrom et al., 1996; Sutton, 1997). Evidence suggests that this is a single dominant gene (LeRoy et al., 1990; Fernandez et al., 1992) and that animals which carry the detrimental allele (RN^-) have increased glycogen levels within the muscle, which is transformed into lactate postmortem resulting in a lower than normal ultimate pH. This so-called acid meat has paler muscle color, increased drip loss and lower processing yield. Currently, the acid-meat condition has only been found within purebred Hampshire populations or populations with Hampshire ancestry, however no estimates of the RN^- allele frequency are available for U.S. populations. Within the United States pork industry, the Hampshire is used widely as a terminal sire and an estimate of the Napole gene frequency will indicate the potential magnitude of the effects of this gene within the industry. The objective of this study was to estimate the Napole gene frequency in a sample of Hampshires comprising several genetic lines provided by a number of influential independent breeders. In addition, carcass and meat quality traits were measured in this population in comparison with a sample of purebred Yorkshires.

Materials and Methods

Trial design. A total of 230 Hampshire-sired pigs were obtained from 23 breeders. Twenty-six of the pigs were crossbred and the remainder were purebred and 43 sires and 152 dams were represented. A sample of purebred Yorkshire (n = 24) from a herd at the University of Illinois were also used in the study. Animals (barrows and gilts) were selected at a minimum live weight of 70 kg and delivered by the individual breeders to the test station at Western Illinois University, where they were penned in like-sire, like-breeder pens. During the study, pigs were housed in a controlled environment finishing house which had partially slatted floors. Animals were given ad libitum access to a finisher diet based on corn and soybean meal and had unlimited access to water. Pigs were reared to approximately 110 kg live weight at which stage they were transported to the slaughter plant of Farmland Inc., Monmouth, IL on the afternoon prior to slaughter and held in lairage for 16 h without food but with access to water. Slaughterings took place on three occasions during the study.

Biopsy sampling. Pigs were allowed a one-week acclimation period after delivery to the test station before biopsies were taken. During the biopsy procedure, pigs were held in a full-sized portable crate scale (Adrian J. Paul Co., Duncan, OK) and a muscle sample was obtained using spring loaded biopsy equipment (Biotech PPB-U, Nitra, Slovakia) with the depth set to penetrate

5.8 cm and samples being taken at a position 5 cm from the midline at the last rib from the right side of the animal.

Glycolytic potential determination. All biopsy samples had the fat and skin portion of the sample removed leaving only muscle tissue. A fresh sample weight was recorded and the lean muscle tissue was then be placed in a 2.0 ml cryogenic vial (Corning Costar Corporation, Cambridge MA), which was stored immediately in liquid nitrogen and transported to the Animal Sciences Laboratory at the University of Illinois. Vials were placed in the freeze dryer for approximately 5-6 days. After removal from the dryer assays were performed on the samples to determine glycogen, glucose, glucose-6-phosphate, and lactate concentrations using standard procedures.
Glycolytic potential values were calculated using the formula described by Monin and Sellier (1985):
Glycolytic Potential = 2 ([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate]

Estimation of allele and genotypic frequency. To estimate the allele and genotypic frequency within this population of American Hampshires, the Hardy-Weinberg equillibrium was used. This is based on a number of assumptions, including 1) the population sampled from is large, and 2) random mating has taken place. Other assumptions used were that the RN gene is a single gene (LeRoy et al., 1990; Fernandez et al., 1992), it is a dominant, two allele system (LeRoy et al., 1990; Fernandez et al., 1992) which is located on an autosomal locus (Milan et al., 1996; Mariani et al., 1996).

Carcass and meat quality evaluation. Carcass data collected include carcass length and last rib backfat thickness measured on the midline. The longissimus muscle from the right side of each pig was removed from the carcass at 24 h post mortem and transported to the University of Illinois Meat Science Laboratory of the University of Illinois for further analysis. Ultimate pH was determined by removing a muscle sample (approximately 3 g) taken at the level of the 10th rib with a no.3, 8 mm cork bore and homogenizing in 10 ml of iodacetate. The pH of the sample was determined using an Orion model 720A pH meter fitted with a Ross Sure Flow 81-72 electrode (Orion Research, Boston, MA). Subjective color, firmness, and marbling were evaluated using the procedures described by NPPC (1991). Objective color (L*, a*, and b*) was measured on the cut surface of the longissimus by using a Hunter LabScan Spectrocolormeter (Model XE, Hunter Associates Laboratory, Inc., Reston, VA). Three loin chops (2.5 cm thick) were taken from the longissimus. One chop was weighed, placed in a Whirl-pak bag, and suspended in a 4°C cooler for 24 h then reweighed and drip loss recorded. One chop was trimmed of epimysium and external fat, homogenized, then placed in a Whirl-pak bag and frozen (-20°C) for subsequent chemical analysis. The remaining chop was used for glycolytic potential determination to validate the original classification by biopsy sampling using the procedures described above.

Chemical analysis. Fat and water contents were determined using the procedures described by Novakofski et al. (1989). All samples were oven dried (110°C for 48 h) and fat was extracted using a mixture of warm chloroform and methanol (4:1).

Results and Discussion

The frequency distribution of glycolytic potential values based on 230 pure or crossbred Hampshire animals is presented in Figure 1. Visual inspection of the distribution suggests that the breakpoint between the two parts of the bimodal distribution is at approximately 185 umol/g. Animals (n = 198) with glycolytic potential greater than 185 umol/g (x = 238.5 umol/g; SE = 2.04) were considered to have at least one copy of the dominant RN^- allele, with animals (n = 32) with glycolytic potential less than 185 umol/g (x = 145.7 umol/g; SE = 5.03) were considered to be homozygous normal (rn^+rn^+) . On this basis, the Yorkshires (n = 24) which were included as a control were considered to be homozygous normal with mean glycolytic potential levels of 146.0 umol/g (SE = 5.98) and a range from 101 to 172 umol / g (Table 2). The difference between glycolytic potential levels of the RN^-RN^- / RN^-rn^+ and rn^+rn^+ Hampshires was 92.8 umol /g ($P < .001$; Table 2), which is similar to the differences reported by Estrade et al. (1993) and Fernandez et al. (1992). Using the Hardy-Weinberg equilibrium to determine allelic and genotypic frequencies within the Hampshire sired population resulted in frequencies for dominant RN^- allele and the normal rn^+ allele of .627 and .373, respectively, and genotypic frequencies .393 for RN^-RN^-, .468 for RN^-rn^+, and .139 for rn^+rn^+ (Table 1). Lundstrom, (cited by Fernandez and Monin, 1994), and Enfalt et al. (1994; 1997) reported frequencies for the dominant allele of .50, .72, and .61, respectively for European populations of pure or crossbred Hampshires.

The carcass traits of the three genotypes are presented in Table 2. Carcass length, was greater for Yorkshires compared to both Hampshire phenotypes (Table 2; $P < .05$). However, the Hampshires were not different between phenotypes, which is in agreement with LeRoy et al. (1996) and Enfalt et al. (1997). The least square means for meat quality measurements and longissimus chemical composition are presented in Table 3. Objective color measurements indicated no differences between Hunter L* values and a* values for the two classifications of Hampshire sired pigs, but Yorkshires had higher L* values ($P < .05$) and lower a* values ($P < .001$) when compared to the Hampshires. However, it is important to note that the Yorkshire population used within this experiment was a limited sample from one herd. Hampshire progeny with the RN^- allele had significantly lower longissimus marbling scores and ultimate pH values when compared to rn^+ Hampshire progeny and Yorkshires ($P < .001$), which is similar to the results reported by several authors (Monin and Sellier, 1985; Enfalt et al., 1994; LeRoy et al., 1996; Lundstrom et al., 1996; Enfalt et al., 1997; Sutton, 1997). As muscle pH approaches the isoelectric point for protein (around pH 5.2), its water holding capacity is dramatically lowered. Water holding capacity, as measured by drip loss ($P < .01$) and cooking loss ($P < .001$), was significantly poorer in RN^- Hampshires compared to the other two genotypes. Several authors have reported increased drip loss (Enfalt et al., 1994; Lundstrom et al., 1996; Enfalt et al., 1997; Sutton, 1997) and cooking loss (Monin and Sellier, 1985; Enfalt et al., 1994; LeRoy et al., 1996; Lundstrom et al., 1996; Sutton, 1997; Enfalt et al., 1997), for animals with high glycolytic potential.

Pigs with the RN^- allele had lower longissimus fat percent ($P < .001$) and higher moisture content ($P < .001$) compared to Yorkshires, with rn^+ Hampshires being intermediate (Table 3). In addition, longissimus protein content was 1.1 percentage unit lower for RN^- Hampshires when compared to rn^+ Hampshires and Yorkshires ($P < .001$). Sutton, (1997) reported a .79 percentage

unit decrease in longissimus protein for RN^- Hampshires compared to rn^+ Hampshires which had normal glycolytic potential levels. Shear force values were not different across the genotypes (Table 3), which is in contrast to the studies of Enfalt et al. (1994), Lundstrom et al. (1996) and Sutton (1997), who reported .2 to .3 kg lower values, indicating a potential tenderness advantage for animals that carry one or two copies of the RN^- allele.

Implications

The high frequency of the RN^- allele and its negative effects on water holding capacity is of concern because of the widespread use of the Hampshire breed in the U.S. Selection against the allele is likely to have a positive impact on processing and curing yields.

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Table 1. Allelic and genotypic frequencies for the Napole gene in American Hampshire and Yorkshire populations.

	Allelic Frequency		*Genotypic Frequency*		
Traits	RN^-	rn^+	RN^-RN^-	RN^-rn^+	rn^+rn^+
Hampshire	.627	.373	.393	.468	.139
Yorkshire	.00	1.00	.000	.000	1.000

Table 2. Least square means for glycolytic potential and carcass measurements.

	Genotype						
	Hampshire sired						
Traits	*RN⁻ -*[d]	*Std Err*	*rn⁺rn⁺* [e]	*Std Err*	*Yorkshire*	*Std Err*	*Sig*[f]
Number of animals	198		32		24		
Glycolytic potential, umol/g							
Biopsy	238.5 [b]	2.04	145.7 [a]	5.03	146.0 [a]	5.98	***
Postmortem	224.7 [a]	2.15	165.5 [b]	5.27	141.5 [c]	6.10	***
Carcass measurements:							
Final live weight, kg	113.9	.52	115.3	1.28	115.5	1.50	NS
Length, cm	83.2 [a]	.16	83.2 [a]	.41	84.5 [b]	.47	*
Last rib backfat, cm	2.53	.043	2.43	.112	2.72	.116	NS

[a.b.c] Means with different superscripts are significantly different ($P < .05$)
[d] Hampshire sired pigs with glycolytic potential > 185 umol / g (includes both purebred and crossbred animals)
[e] Hampshires sired pigs with glycolytic potential < 185 umol / g (includes both purebred and crossbred animals)
[f] NS, *, *** = not significant, $P < .05$, $P < .001$, respectively

Table 3. Least square means for meat quality and chemical composition measurements taken on the longissimus.

	Genotype						
	Hampshire sired						
Traits	*RN⁻* [d]	*Std Err*	*rn⁺rn⁺* [e]	*Std Err*	*Yorkshire*	*Std Err*	*Sig* [f]
Meat quality:							
Hunter L*	49.7 [a]	.27	49.4 [a]	.66	51.7 [b]	.79	*
Hunter a*	7.8 [a]	.08	7.5 [a]	.20	6.8 [b]	.24	***
Hunter b*	16.1	.09	16.0	.23	16.0	.27	NS
Color	2.2	.05	2.4	.12	2.2	.14	NS
Firmness	1.8	.05	1.7	.11	2.1	.13	NS
Marbling	2.2 [b]	.05	2.6 [a]	.14	2.7 [a]	.16	***
Drip loss, %	5.87	.191	4.32	.471	4.58	.549	**
Ultimate pH	5.42 [b]	.006	5.52 [a]	.016	5.55 [a]	.019	***
Cooking loss, %	25.5 [b]	.35	21.2 [a]	.86	22.7 [a]	1.00	***
Shear force, kg	2.85	.040	2.99	.097	3.02	.115	NS
Chemical composition, %:							
Protein	21.9 [b]	.09	23.0 [a]	.21	23.4 [a]	.25	***
Moisture	75.0 [a]	.07	74.5 [b]	.17	73.3 [c]	.19	***
Fat	1.82 [a]	.05	2.11 [a]	.13	2.68 [c]	.15	***

[a,b,c] Means with different superscripts are significantly different (P < .05)
[d] Hampshire sired pigs with glycolytic potential > 185 umol / g (includes both purebred and crossbred animals)
[e] Hampshires sired pigs with glycolytic potential < 185 umol / g (includes both purebred and crossbred animals)
[f] NS, *, *** = not significant, P < .05, P < .001, respectively

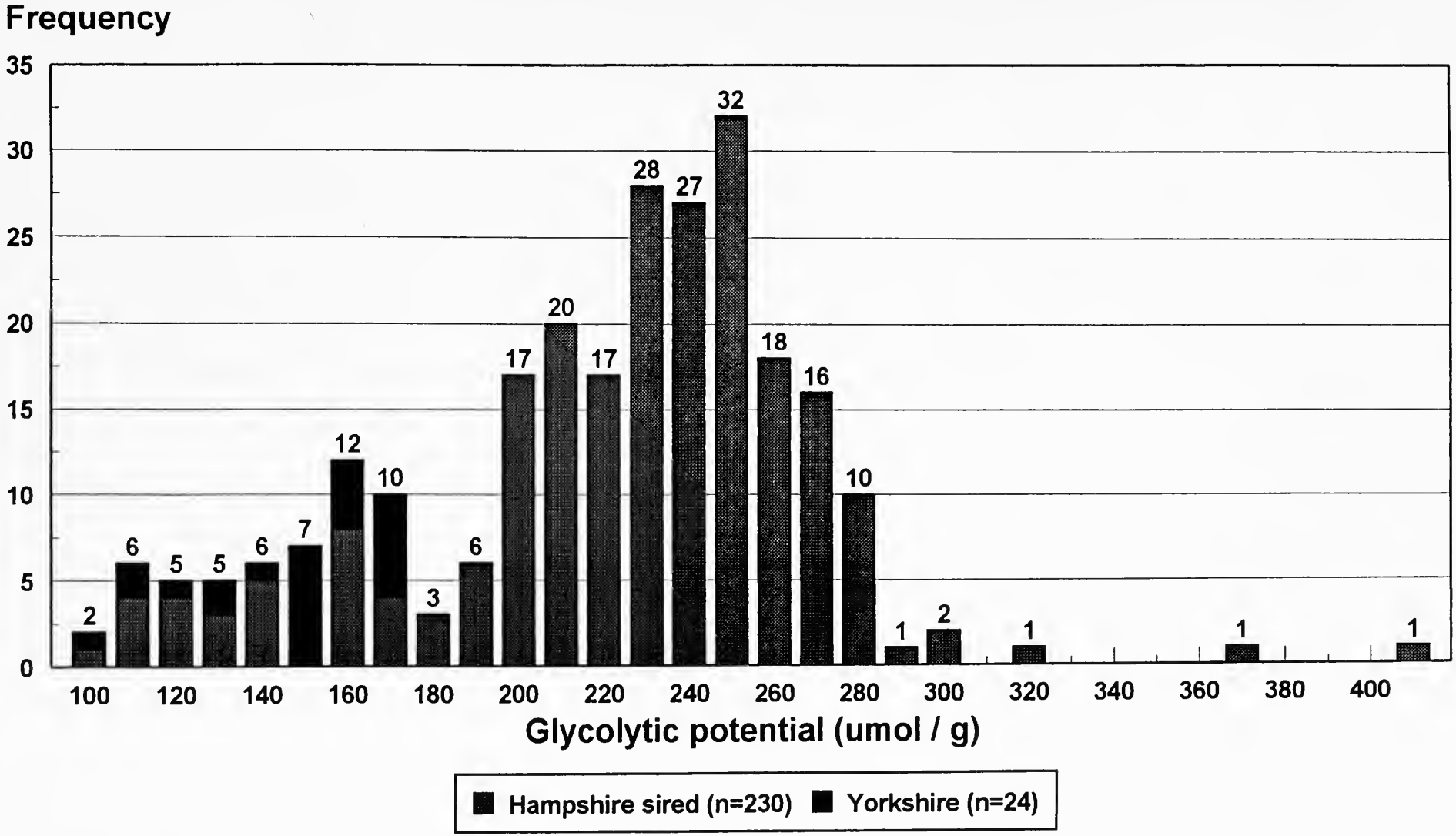

Figure 1. Distribution of live-animal longissimus glycolytic potential values determined from biopsy samples

INTERACTION OF NURSERY AND GROW-FINISH SPACE ALLOCATION

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Introduction

This study was carried out as part of a collaborative project coordinated by the NCR-89 Committee on Swine Management. The results for the portion of the study carried out at the University of Illinois are reported here.

Space restriction has been shown to decrease daily gain and feed intake in pigs (Brumm and Miller, 1996; Kornegay and Notter, 1984), but has given variable results for gain to feed ratio (Moser et al., 1985; Randolph et al., 1981; Brumm and Miller, 1996). Space allowance for pigs has been investigated in conjunction with other factors. Gonyou and Stricklin (1998) and Gehlbach et al. (1966) studied the interaction of space allowance and group size. Kornegay, Lindemann, and Ravindran (1993) and Brumm and Miller (1996) studied the interaction of nutrition and space allowance, and Moser et al. (1985) investigated the interaction of a feed additive and space allowance. Other studies have investigated the effects of space allowance with nursery pigs (Kornegay, Lindemann, and Ravindran, 1993; NCR-89, 1989) and grow-finish pigs (Brumm and NCR-89, 1996; Meunier-Salaun et al., 1987; Gonyou and Stricklin, 1998). These studies all investigated the effects of space allowance in one phase of the production cycle only. Brumm and Dahlquist (1995) investigated the interaction between space allowance in the nursery and in the grow-finish phases of production. They found that pigs that were crowded in the nursery showed a reduced response to crowding in the grow-finish compared to those that were not crowded in the nursery.

The objective of this study was to follow up on this initial finding and study the interaction between space allowance in the nursery phase and grow-finish phase on pig performance from weaning to market weight.

Experimental Procedure

Ninety pigs (n=90) of a commercial hybrid genotype (PIC 326 sire x C-22 dam) were randomly allotted to treatments based on litter of origin, weight, and sex. A 2 x 2 factorial arrangement was utilized to investigate the effects of two nursery space allowances and two grow-finish space allowances. Three replicates of the study were conducted. Weaned pigs were housed at either 1.75 ft^2/pig (crowded) or 2.50 ft^2/pig (uncrowded), with two pens per space allowance per replicate. Pen size was kept constant across space allowances in the nursery, with the two space allowances being achieved by varying group size between 6 and 9 pigs. Upon transfer to the grow-finish phase, pigs were housed at either 6.5 ft^2/pig (crowded) or 8.0 ft^2/pig (uncrowded).

Although space allowance was confounded with group size (6 and 9), studies investigating group size and space allowance have found no interaction between number of pigs per pen and floor space allowance using group sizes of 5 and 10 pigs (Randolph et al., 1981) or 5 and 30 pigs (Kornegay and Notter, 1984). In the event of pig death or removal from study, pen size was adjusted to maintain the same space allowance per animal.

Nursery Phase

This phase was carried out in a conventional nursery accommodation that was environmentally controlled. Piglets were given *ad libitum* access to feed and water from a four-hole feeder and a nipple drinker, respectively. Pen dimensions were 4 feet by 4 feet. Pigs were weighed at the start of the study and weekly thereafter. Feed intake was measured weekly. Pigs were moved to the grow-finish phase of the study when both pens of uncrowded pigs in each replicate averaged 45 pounds.

Nursery pigs were fed a three-phase diet regime formulated to meet or exceed NRC (1988) requirements for all nutrients.

Data collected for the nursery period included pig weights, feed intake, and days in the nursery, from which average daily gain, average daily feed intake, and gain to feed ratio were calculated.

Grow-finish Phase

Pigs were moved from the nursery to the grow-finish accommodation while maintaining the same pen groups. The grow-finish accommodation was environmentally controlled and had partially-slatted flooring. Pen dimensions, taking into account feeder space, for groups of 6 pigs were 6.6 by 7.5 ft. for uncrowded pens and 5.4 by 7.5 ft. for crowded pens. Pen dimensions for groups of 9 pigs were 9.8 by 7.5 ft. for uncrowded pens and 8.0 by 7.5 ft. for crowded pens. Pigs were offered *ad libitum* access to a corn/soybean meal diet. Diets were formulated to contain 1.0% lysine for the first week in the grow-finish phase; 0.95% lysine for pigs up to 80 pounds; 0.85% lysine for pigs between 80 and 130 pounds; 0.75% lysine for pigs between 130 and 190 pounds; and 0.60% lysine for pigs between 190 pounds and slaughter (240 pounds). Diets were formulated to meet or exceed NRC (1988) requirements. Diet phases were switched as pen average weights reached the predetermined levels. Each pen contained a two-hole feeder and one nipple drinker. Pen sizes were adjusted in the event of pig death or removal prior to pigs attaining the target end weight, maintaining the same area per pig and ratio of concrete to slats.

Pigs were taken off test when they reached a target end weight of 240 pounds. If more than 50% of the pigs in a pen had been removed from test, the remainder of the pigs stayed on test until they averaged 240 pounds or for 3 weeks, whichever was sooner. As pigs were removed from test, a longitudinal ultrasound scan anterior to the last rib was taken to determine backfat and loin eye depth, and these measurements were used to estimate the percentage of lean in the carcass. Pigs not reaching a minimum weight of 220 pounds were not scanned.

Data collected for the grow-finish period included weekly pig weights, weekly feed intake until the first pig was removed from test, and death loss. These data were used to calculate average daily gain, average daily feed intake, gain to feed ratio, initial lean composition (based on the

NPPC formula for lean composition of a 40 to 90 pound pig), percent lean at end, average daily lean gain, and lean gain efficiency.

Statistics

Data was analyzed using the GLM procedures of SAS (1990), using the pen as the experimental unit. The model used included the effects of space allowance treatments in the nursery and grow-finish phase and the interaction.

Results and Discussion

Nursery Phase

Treatment means for the nursery phase are reported in Table 1. Crowding (1.75 ft^2 space allowance) reduced average daily feed intake ($P<.05$) by 12.5% and numerically decreased average daily gain ($P=.065$) by 9.0%. However, the crowding treatment did not affect end weight or gain to feed ratio, which is in agreement with NCR-89 (1989) which reported crowding at 1.33 ft^2/pig, compared to 2.66 ft^2/pig, significantly decreased feed intake by 11.2% and daily gain by 11.0%, with no affect on gain to feed ratio. In another study, crowding pigs (1.5 ft^2/pig) compared to giving the pigs a greater space allowance (3.0 ft^2/pig) reduced average daily feed intake by 11.6% and average daily gain by 12.1%, with no effect on gain to feed ratio (Kornegay et al., 1993). Thus, the results of the current study, which compared space allowances of 1.75 ft^2/pig compared to 2.50 ft^2/pig, caused reductions in pig performance comparable with the studies reported above.

Grow-Finish Phase

Treatment means for grow-finish performance reported by nursery treatment and grow-finish treatment are presented in Table 2. Nursery treatment did not influence growth performance in the grow-finish stage.

In addition, crowding in the grow-finish phase did not affect daily feed intake and daily gain at this stage of production. However, crowding tended to reduce overall gain to feed ratio ($P=.065$). Grow-finish treatment did not affect any other performance measures. Other studies have indicated significant treatment differences in pig performance between crowded and uncrowded conditions. Brumm and Miller (1996) studied space allowances of 6.1 ft^2/pig (crowded) and 8.4 ft^2/pig (uncrowded) and showed a 4.8% reduction in average daily gain and a 6.2% reduction in average daily feed intake, with no difference in gain to feed ratio. Brumm and NCR-89 (1996) investigated space allowances of 7.0, 9.0, and 11.0 ft^2/pig and showed that as space allowances decreased from 9.0 to 7.0 ft^2/pig, average daily gain was reduced 1.6% and gain to feed ratio was reduced 1.2%. Moser et al. (1985) investigated the influence of space allowances of 3.0, 3.6, and 4.0 ft^2/pig for growing pigs between 23 and 55 kilograms, and 6.0, 7.1, and 8.0 ft^2/pig for finishing pigs between 55 and 100 kilograms. Average daily gain and gain to feed ratio for finishing pigs were reduced by 7.0 and 3.5%, respectively, when space allowances were reduced from 8.0 ft^2/pig to 6.0 ft^2/pig (Moser et al., 1985). The space allowances in the grow-finish phase of this study of 6.5 and 8.0 ft^2/pig are similar to those presented above. However, in the current study, restricting space allowance of pigs to 6.5 ft^2/pig did not reduce growth performance.

Interactions between nursery and grow-finish space allowance treatments were not significant for any performance measure, and therefore, the interaction means are not presented. Thus, in the current study, the floor space allowance in the nursery had no impact on the subsequent performance of the pigs. This is in contrast to the findings of Brumm and Dahlquist (1995) who found an interaction between nursery and grow-finish space allowance for growth performance in the grow-finish phase. In grow-finish, pigs in crowded pens actually grew faster (+4%) than uncrowded pigs if they had been crowded in the nursery, but grew slower (-9%) if they were from uncrowded nursery pens. This indicates that pigs crowded in the nursery were less affected by crowding in the grow-finish than those pigs given ample space in the nursery. The space allowances used for crowded and uncrowded pigs in the current study (1.75 and 2.50 ft^2/pig in the nursery and 6.5 and 8.0 ft^2/pig in the grow-finish, respectively) were similar to those used by Brumm and Dahlquist (1995) of 1.8 and 2.7 ft^2/pig in the nursery and 6.1 and 8.4 ft^2/pig in grow-finish, respectively.

Conclusions

1. Crowding pigs in the nursery at 1.75 ft^2/pig, compared to 2.50 ft^2/pig, reduced average daily feed intake by 12.5% and average daily gain by 9.0%; but crowding pigs at 6.5 ft^2/pig, compared to 8.0 ft^2/pig, in grow-finish did not affect pig performance at this stage of production.
2. There was no interaction between crowding in the nursery and grow-finish phases.
3. This suggests that decisions regarding floor space allowances in nursery and grow-finish phases of production can be made independently.

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Table 1. Least squares means for nursery phase by treatments.

	Nursery Treatment		
Trait	*Uncrowded*	*Crowded*	*Ave. SE*
Start Weight, kg	4.7	5.0	0.4
End Weight, kg	21.9	20.8	0.85
Average daily gain, kg	0.41	0.38	0.012
Average daily feed intake, kg	0.62[a]	0.54[b]	0.025
Gain:Feed	0.68	0.70	0.023

[ab] Means with different superscripts differ (P<.05)

Table 2. Least squares means for pig performance in the grow-finish phase and wean to market by nursery and grow-finish treatments[a].

	Nursery treatment			*Grow-finish treatment*		
Trait	*UCR*	*CR*	*Ave SE*	*UCR*	*CR*	*Ave SE*
Performance in Grow-Finish:						
Start weight, kg	22.0	20.5	0.35	21.5	21.0	0.35
End weight, kg	110.6	107.5	1.13	108.5	109.7	1.14
Average daily gain, kg	0.85	0.81	0.024	0.84	0.82	0.024
Average daily feed intake, kg	2.42	2.32	0.061	2.37	2.38	0.061
Gain:feed	0.35	0.35	0.005	0.35	0.35	0.005
End lean, %	53.25	52.41	0.320	52.38	53.29	0.320
Average daily lean gain, kg	0.36	0.36	0.004	0.36	0.36	0.004
Lean gain:feed	0.15	0.16	0.005	0.15	0.15	0.005
Performance from Weaning to Market:						
Average daily gain, kg	0.73	0.68	0.016	0.71	0.70	0.016
Average daily feed intake, kg	1.89	1.80	0.042	1.82	1.86	0.042
Gain:feed	0.39	0.38	0.002	0.39	0.38	0.003

[a] UCR = Uncrowded (2.5 ft^2/pig in nursery, 8.0 ft^2/pig in grow-finish)
CR = Crowded (1.75 ft^2/pig in nursery, 6.5 ft^2/pig in grow-finish)

Cellular Uptake of Amino Acids by Lactating Sow Mammary Tissue

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Introduction

Growth of the newborn pig is dependent upon the sow. The sow must be able to produce a quantity and quality of milk that meets the demands of the growing litter. Improved nutrition for the high-producing lactating sow is required to reap the full benefits of advances in sow productivity. Efficiency of milk protein synthesis is determined by plasma concentration, cellular uptake, and intracellular metabolism of amino acids.

The lactating mammary gland takes up free amino acids from the blood in large quantities to satisfy the needs of protein synthesis. Mammary arteriovenous differences of amino acids demonstrate the importance of essential amino acids to mammary tissue (Trottier et al. 1997). Lysine is taken up in amounts adequate to account for its appearance in mammary protein, while valine and other branched chain amino acids are taken up in excess of requirements for milk protein synthesis. A proportion of amino acids are also used for synthesis of cellular protein or are oxidized in mammary cells. These alternate uses of amino acids by the gland generally are not taken into account in whole animal nutritional studies. Nutrition studies indicate that several amino acids other than lysine may be limiting to sow lactation performance and that feeding supplemental dietary lysine may alter the balance of amino acids in the blood and alter mammary uptake (Knabe et al., 1996; Richert et al., 1996).

To better understand the process of milk protein synthesis, it is necessary to gain a basic knowledge of the individual transporters for cellular uptake and their regulation. Amino acid transporters may be a rate-limiting step in milk synthesis and understanding of how they function could be used to manipulate milk output. By optimizing amino acid concentration through sow nutrition, pigs may grow faster and have a higher survival rate. At least five distinct transport systems have been characterized that transport amino acids into the mammary secretory cells (Shennan 1998). These transporters are on the blood-facing membranes of alveolar cells. The transporters have been classified based upon ion dependency and substrate specificity. Most of this work has been done in ruminants and rodents. However, the amino acid uptake mechanisms and intracellular metabolism have not been characterized for the lactating sow. Extrapolation of knowledge from other tissues or from mammary gland of other species may not be directly applicable to the lactating sow.

The amino acid uptake systems can be broadly classified by ion dependency. Some amino acids are taken up in excess of their appearance in milk and plasma, which suggests an input of energy during uptake. A sodium gradient across the membrane may drive the transport in these systems.

In the mammary gland, several sodium dependent systems have been identified and are generally referred to as systems A, ASC, and X_{AG}.

System A (identified in mouse, rat, and bovine mammary tissue) prefers neutral amino acids for substrates, especially short-chain amino acids (alanine, methionine, glycine, proline). System A appears to be physiologically regulated, having an increase in activity from pregnancy to lactation in mouse mammary tissue.

System ASC transports the neutral amino acids (cysteine, serine, alanine, threonine, methionine). There is substrate overlap between ASC and system A. The ASC system has been identified in ruminants and guinea pigs, though there is little evidence for an ASC transport system separate from system A in the mouse or rat, demonstrating a significant variation in mammary tissue among species.

System X_{AG}, an anionic amino acid transport system, is responsible for the uptake of glutamate, aspartate and histidine. Glutamate accounts for a large percentage of the milk protein bound amino acids. System X_{AG} may be responsible for meeting the large requirements of the mammary gland for glutamate.

Two sodium independent systems have been identified in mammary tissue. System L, as identified in mouse, rat, guinea pig and cow, is principally responsible for the uptake of the branched chain amino acid (valine, leucine, isoleucine), but also allows uptake of all the neutral amino acids. The versatility of this transporter makes it one of the most important transport systems in mammary tissue.

A cationic amino acid (lysine, arginine, ornithine) uptake system, the Y^+ system, also has been described in the rat and cow. Lysine is taken up in high quantities by the lactating mammary gland. In species such as the cow and the goat it is taken up in excess of its appearance in milk. This cationic uptake system interacts with certain neutral amino acids as demonstrated in the rat. However, the cationic uptake system in the mammary gland is not the classical Y^+ system observed in other epithelial tissues, demonstrating again the variation with amino acid transport systems.

The current lack of fundamental information on amino acid uptake systems compromises efforts to make changes in dietary requirement estimates for the economically important sow. The objective of this study is to begin to characterize the specificity of mammary gland uptake systems of amino acids vital to lactating sows.

Experimental Approach

Lactating sows (day 15 of lactation or later) from the University of Illinois swine herd were slaughtered at the University of Illinois Meat Science Laboratory abattoir. Litters were removed immediately prior to slaughter. Mammary tissue fragments were incubated in a medium containing a low concentration of the test amino acid and supraphysiological concentrations of competitors to characterize the specificity of the respective uptake systems.

Preliminary Results

Preliminary studies of lysine with competitors have shown lysine uptake to be consistent with the Y^+ transport system as the high affinity uptake mechanism (Table 1). Arginine and ornithine (cationic amino acids which use the Y^+system) are strong inhibitors of lysine uptake. In addition to the cationic amino acids, the neutral amino acids also have a strong interaction with the uptake of lysine. Leucine and cyclo-leucine, both substrates for the L uptake system, showed inhibition of lysine uptake, though not as strong as the cationic amino acids. Other neutral amino acids such as methionine and alanine (system ASC substrates), also inhibited lysine uptake. Lysine uptake was partially inhibited by D-lysine, indicating that the uptake mechanism is only partially stereospecific. Most naturally occuring amino acids are of the L stereoisomer. A substrate for system A, MeAIB (methyl aminoisobutyric acid), did not significantly inhibit lysine uptake.

Conclusion

These results are consistent with lysine uptake in the lactating sow mammary gland by a Y^+-type consistent with lysine uptake in the lactating sow mammary gland by defining basic amino acid uptake kinetics and may provide important information for optimizing amino acid nutrition for lactating sows.

Acknowledgments

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Table 1. Inhibition of lysine uptake in lactating sow mammary tissue by other amino acids.[1]

Amino Acid Competitor	*% Inhibition*[2]
Ornithine	85.70 ± 7.57
Arginine	83.22 ± 8.85
Methionine	52.44 ± 6.58
Alanine	65.24 ± 3.78
cyclo-Leucine	38.57 ± 6.28
Leucine	49.01 ± 6.52
D-Lysine	51.97 ± 8.74
MeAIB[3]	12.69 ± 10.05

[1]Lysine at 20 micromolar and competitors at 20.28 millimolar; n = 6 sows.
[2]Inhibition calculated as uptake in the presence of competitor as a percentage of uptake in the absence of competitor. Values are means ± standard error of the mean.
[3]MeAIB = methyl aminoisobutyric acid

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Influence of Isoflavones on Porcine Embryonic Development and Embryonic Gene Expression

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Project Summary
One of the most critical aspects of swine reproduction is embryonic development. Embryonic development is directly related to litter size, an indicator of the reproductive capacity of a sow or gilt. In pig reproduction, it is known that estrogen plays a major role in early embryonic development and maternal recognition of pregnancy. Maternal recognition of pregnancy is a reproductive process of which little is known. Nonetheless, it is known that a minimum of four embryos must be present in the uterus 12-15 days after fertilization to maintain pregnancy. One of the most important factors for maternal recognition of pregnancy in the pig is the production of estrogen by the embryo. This estrogen production by the embryo prevents PGF_2 alpha from being secreted into the lumen of the uterus. The prevention of PGF_2 alpha secretion allows the embryos to continue to develop. The mechanism for estrogen production by the embryo, however, is unknown. It is not known whether the embryo produces the estrogen from basic precursors or converts another substance into estrogen.

There are many estrogenic isoflavones called phytoestrogens found in the products of soybeans. Presently, the role of the isoflavones in early embryonic development in the pig has not been investigated. Isoflavones may play a role in embryonic development and also gene expression of the early embryos. Gene expression of the early embryo may be important by allowing certain growth factors to be turned on or off to increase embryo survival. An increase in the number of embryos surviving directly increases the litter size and improves reproductive performances. With Illinois being one of the top five hog and soybean producing states in the U.S., it's necessary to conduct the research to determine the role of isoflavones in porcine embryonic development and embryonic gene expression. This research may help increase the consumption of soybean based products in swine diets.

Project Objective
To determine the effects of isoflavones on development in vitro of porcine embryos.

Introduction
Early embryogenesis and factors that affect early embryogenesis are important areas of research at the University of Illinois. Proper embryonic development is important for embryo survival, which directly correlates to litter size and reproductive performance.

Growth factors are critical in the control of animal cell growth (Simmen et al., 1990). The regulation and exposure of growth factors is becoming an important mechanism that needs to be investigated. It is known that growth factors work with the second messenger system, such as cyclic adenosine monophosphate (cAMP), phosphoinsoitol or calcium effector pathway (Hill, 1991). There are intercellular mechanisms involved with growth and maturation of tissues classically controlled by hormones such as estrogen (Simmen et al., 1988).

There has been some investigation into the effects of growth factors on embryo development in the mouse and bovine. The growth factors that have been investigated are insulin-like growth factor II, platelet-derived growth factor, epidermal growth factor, uterine-derived growth factor, and colony-stimulating factor-1. However, there has been some investigation into the effects of

growth factors in swine. Abeydeera et al. (1997) reported that epidermal growth factor had no beneficial effect on blastocyst development in vitro. However, epidermal growth factor was shown to have a beneficial effect on subsequent development of in vitro matured and fertilized pig embryos when added to the maturation medium. Uterine luminal fluid mitogen (ULFM) is a growth factor that promotes DNA synthesis in cultures of porcine uterine cells in domestic pigs and might regulate uterine growth and/or differentiation (Simmen et al., 1988). On day 12 of gestation, insulin-like growth factor I (IGF-I) is at it's highest level and appears to be regulated by estrogen. In the pig embryo, it is not known whether the embryos produces estrogen, acquires the estrogen from the uterine lumen fluid or converts another substance to estrogen. It has been shown that IGF-I promotes protein synthesis in porcine embryonic discs in vitro (Estrada et al., 1991).

Cytokines are signals that act as local chemical mediators of cellular functions. These are important signals in associations such as maternal-embryo association in early pregnancy. Ovine and bovine use cytokines for maternal recognition of pregnancy. Interferon-tau is a cytokine that is produced by the embryo prior implantation to signal the uterus to continue the pregnancy. It has been reported that interferon-tau levels were not different among embryos of different breeds (Bonnardiere et al., 1991). Little research has been done to determine the effects of phytoestrogens on growth factors or cytokines.

Materials and Methods

Prepubertal gilts will be superovulated with an injected PG600® at approximately 200 days of age followed by an injection of human chorionic gonadotropin (hCG). Gilts will be mated to produce hybrid embryos. Two-cell embryos will be collected surgically from the donor gilts. Following collection, embryos will be cultured with and without increasing doses (0, 10nM, 30nM, 100nM, 300nM, 1μM, 3μM) of isoflavones will be added to Whitten's with a charcoal stripped (to remove any exogenous estrogens) protein supplement. The embryos will be cultured for 96 hours and stage of development will be evaluated and recorded every 24 h. As the embryo ceases to develop, it will be fixed in a 3:1 ethanol:glacial acetic acid fixative and stained with aceto-orecin to determine number of nuclei. The number of nuclei will give an index of cell divisions and embryonic development. However, embryos reaching the blastocyst stage will be stained with propidium iodine and Hoechst 33342 to determine the inner cell mass number as well as the total cell number.

Gene expression of embryonic growth factors will be determined by Reverse Transcription Polymerase Chain Reaction (RT-PCR). RNA will be isolated from a subset of embryos developing to the blastocyst stage. RT-PCR will be performed in a thermal cycler at the optimal conditions to obtain cDNA. The cDNA will then be amplified by radioactive PCR and separated on a 6% polyacrylamide gel. After separation, the gel will then be attached to blotting paper and exposed to X-ray film for 19 hours. Following autoradiography, bands of interest will be removed, purified and checked for presence of cDNA by PCR.

Statistical analysis of the isoflavone concentrations on stage of development and cell number will be determined by analysis of variance (ANOVA). To compare growth factor dose-response curves, more precisely, the 4-parameter logistic fit model of David Rodbard, NIH (DeLean et al., 1978) adapted to IBM-PC will be used. This program permits simultaneous analysis of families of sigmoidal curves with statistical comparisons of maximal and ED50's doses (concentrations effectively stimulating half-maximal response). The ED50's will be compared using Student's T-test. Data will also be analyzed by ANVOA with stage of development, cell number and growth factor expression as the main effects.

Conclusions

The use of isoflavones to increase porcine embryonic development and gene expression could have a profound effect on Illinois agriculture. This research affects both the hog producer as well as the grain producer. A soybean price increase driven by a higher demand for soybean by-products

used in swine diets will benefit the grain producer. Also, an increase in embryo survival will increase the efficiency of hogs production, benefiting the hog producer. Isoflavone could have the potential of having a profound effect on Illinois as well as U.S. agriculture.

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Lichtensteiger, Carol	Diagnostic Pathology	217-333-2449
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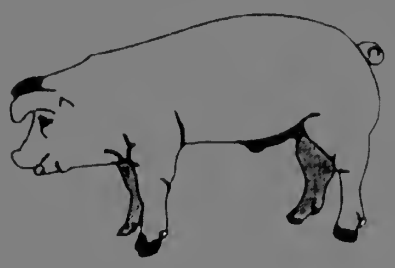

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Introduction

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Establishing Nutrient Requirements for the Lactating Sow: A Summary of Recent Illinois Research

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Introduction

Genetic improvement of sows has been significant during the past two decades. Modern sows are not only leaner and heavier at maturity but also produce larger litters. A simple comparison of 1979 British national data (MLC, 1979) with similar data (MLC, 1998) 20 years later reveals a three-pig per year increase in pigs weaned per sow per year. Nutritionists are challenged to accurately estimate the nutrient needs of contemporary sows. Most efforts to date have been based on experimental results where birth weight, litter size, milk yield of the sow, and body condition of the sow have been measured in response to specific dietary treatments. We believe that further progress can be made by understanding how nutrients flow from the diet to storage and productive tissues in the body and how nutrients are mobilized from internal tissues and organs during periods of great nutrient demand such as lactation.

Conventional wisdom and some experimental data suggest that modern sows have less appetite in comparison to the animals that existed twenty or thirty years ago. At the same time, an improved capacity for milk production and the demands for milk by larger litters require that the sow either eat more or accelerate the extraction of nutrients from body tissues. The problem appears to be particularly acute in the lactating first-litter gilt. Inadequate feed intake during lactation affects return to estrus and subsequent litter size . Primiparous sows, bred younger than in the past, require nutrients for growth in addition to the demands for development of the conceptus and, post-partum, for milk production.

Models

Models are representations of reality. Good models facilitate the rapid comparison of a number of different scenarios. One approach to understanding sow nutrition is through the use of computational models that give considerations to key nutritional inputs and outputs. In order to develop such a model we began a series of experiments in the early 1990's designed to provide

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empirical data to use in the construction of a model of nutrient flow in the lactating sow. Specifically, we sought to understand: (1) the flow of nutrients among different organs and tissues during lactation, (2) the role of specific amino acids in controlling feed intake during lactation, (3) the appropriate approach for estimating availability of dietary amino acids for the lactating and gestating sow, (4) amino acid uptake by lactating mammary glands and subsequent utilization of nutrients for glandular growth and milk production and, (5) the effect of teat order, mammary capacity and nursing behavior on piglet growth. Using these data we created a dynamic simulation model to predict size and composition of organ tissues, growth of mammary glands and nursing pigs, and carcass size and composition in response to changes in diet composition, length of lactation, feed intake, and litter size.

Now, we will briefly summarize each of the projects.

Feed Intake

Ultimately, nutrient requirements for lactating sows must include consideration of the sow's capacity for intake. Poor appetite is considered a constraint on performance in lactating primiparous sows (Lynch, 1988). Body condition at farrowing (O'Grady et al., 1985) and weight gain during gestation (Weldon et al., 1994) influence feed intake during lactation. Overfeeding sows during gestation can lead to excessive body fat and, consequently, low feed intake during lactation.

Trottier (1996) in our lab used primiparous sows to examine the relationship between low feed intake and increased weight loss during lactation and the alternative, increased gestation fat gain due to high energy intakes (Figure 2 and 3). Metabolic hormones were of particular interest. Plasma insulin and glucagon levels in primiparous sows fed a low-energy diet during gestation were higher than in counterpart sows fed a high energy diet (Figure 4). High insulin and low glucagon levels may reduce nutrient mobilization from body tissues and thus, increase appetite of the sows. Sows with a high energy intake during gestation exhibit low plasma insulin and elevated glucagon concentrations during early lactation. This is consistent with poor appetite during lactation.

Feed intake during gestation should be high in order to maximize protein retention, yet directed to achieve optimum, not maximum, fat accretion. This strategy would maximize insulin release, minimize glucagon levels and, as a result, increase voluntary feed intake during lactation.

Digestibility of Amino Acids in Major Feed Ingredients by The Lactating Sow

It is essential to know the extent to which the sow can extract nutrients from the diet that is provided. To date, the most widely utilized method for estimating nutrient digestibility is based on samples taken from the end of the small intestine, i.e., the terminal ileum. However, the digestibility values present in the scientific literature are based on samples taken from growing pigs, not from lactating sows. These digestibility values from growing pigs have been used for modeling nutrient requirements for lactating sows (Whittemore and Morgan, 1990; Pettigrew et

al., 1992). Are digestibility values obtained with growing pigs representative of actual nutrient extraction in the sow? That hypothesis was tested in our next experiment.

Stein et al. (1999a) obtained ileal digestibility values for protein and amino acids for six feed ingredients, corn, soy bean meal, barley, wheat, canola meal, and meat and bone meal, in both growing pigs and sows during gestation and lactation (Table 1). The results indicate that lactating sows have higher apparent ileal digestibility values for most amino acids than growing pigs. This maybe because of elevated secretions of digestive enzymes or longer digestive tract. A reduced loss of endogenous protein at the distal ileum (Stein et al., 1999b) could be an another reason for the higher apparent ileal digestibility values obtained in lactating sows than in growing pigs. Gestating sows had a lower apparent ileal digestibility values than lactating sows.

Nutrient Mobilization in Lactating Sows

Dietary nutrients are often insufficient to support milk production during lactation, especially in primiparous sows. Fortunately, sows are able to adapt to this situation by mobilizing nutrients from body tissues. Organs can become significant sources of nutrients and each organ may respond differently. Escobar (1998) and Kim (1999) investigated nutrient mobilization from different tissues during a 28-day lactation. On a percentage basis, the reproductive tract had the highest level of nutrient mobilization (Escobar, 1998). However, this is undoubtedly associated with normal postpartum regression. Kim (1999) demonstrated that the additional protein mobilization associated with an larger litters occurred primarily from the reproductive tract. Interestingly, fat and protein mobilization occurred independently of each other in the different tissues (Escobar, 1998).

Nutrient Use by the Mammary Gland during Lactation

It is essential that lactating sows provide adequate milk to nursing pigs. Thus, increasing milk yield has been one of the primary objectives for nutritionists. However, nutrient uptake and utilization by the porcine mammary gland has received little research attention. Concepts are largely based on extrapolations from studies with lactating cows.

Recent studies from our lab provide new information about sow lactation. The quantity of nutrients taken up from circulating blood by mammary glands was measured (Trottier et al., 1997; Nielsen et al., 1997) and the amount of nutrients used for mammary tissue growth was characterized (Kim et al., 1999a, b, and c).

In order to quantify nutrient uptake by mammary glands, it was necessary to develop procedure for measurement. Trottier et al., (1995) surgically fitted catheters in both the mammary vein and the carotid artery as a means of comparing arterial and venous concentrations. Using these comparisons the investigators found that almost 180 g of essential amino acids were taken up each day by mammary glands and of these 49 g were not secreted as a milk. These amino acids were apparently retained in the mammary gland (Table 2) for other functions. Thus, it was

apparent to us that estimating amino acid needs based on output in milk can underestimate reality.

Kim et al. (1999a) conducted a follow-up experiment to investigate the amount of nutrients accumulated as a consequence of mammary tissue growth during lactation. That work indicated that there is a daily accumulation of seven grams of essential amino acids in sows nursing 10 pigs (Table 2). This amount represents 14.3% of essential amino acids that are taken up by the glands after milk secretion.

We have also attempted to characterize how mammary gland metabolism responds to litter size (Nielsen et al., 1997; Kim et al., 1999b). Experiments were conducted using sows nursing different numbers of piglets. Approximately 19 g of additional essential amino acids were taken up by mammary glands daily for each nursing pig during the 21-d lactation. The amount of essential amino acids additionally accumulated as mammary tissue proteins was .83 g daily with an additional nursing pig during 21-d lactation. When it is considered that 14.3% of essential amino acids are retained in the mammary glands, the amount of essential amino acids used for mammary gland growth for nursing pig is 5.8 g per day. Thus, it can be predicted that sows with 10 nursing pigs need 49 g of essential amino acids each day for mammary gland growth (7 g accumulation) and as litter size increase sows need additional 5.8 g essential amino acids daily per nursing pigs (.83 g additional accumulation).

Amino acid needs of the mammary gland for growth have not been considered until now in the development of predictive models. As a result, the actual daily amino acid needs may be greater than predicted by the current NRC (1998) model. To test this hypothesis we conducted a practical study to determine the quantity of total daily amino acid intake that would maximize mammary gland growth (Kim et al., 1999c). Growth was maximized when sows received 55 g lysine daily; 8 g more than predicted by the NRC (1998) model for these sows. The studies from our lab (Kim et al., 1999a, b, and c) have indicated that consideration of mammary growth needs can contribute to better estimation of nutritional needs for lactating sows.

Summary and a Dynamic Model Nutrient Flow in Lactating Sows

Providing optimum intake of nutrients to sows is essential if sow productivity is to be maximized and feed costs minimized. Establishing accurate nutrient requirements in lactating sows is not an easy process; it is a complex system. Understanding the flow of nutrients in the sow during lactation provides a basis for establishing nutrient requirements. Nutrient flow among various organs can be computed using our dynamic simulation model. Linear or nonlinear regression models were developed to relate information from the projects described above to create a dynamic model that simulates nutrient flow in sows during lactation (Kim et al., 1999d). Information used to construct the model included: apparent ileal digestibility of various protein sources in lactating sows (Stein et al., 1999) and nutrient mobilization in the sow's body as influenced by feed intake (Escobar, 1998) and litter size (Kim et al., 1999b), nutrient uptake by mammary gland (Trottier et al., 1997), nutrients need for mammary gland growth (Kim et al., 1999 a and c), and the growth of nursing pigs in response to maternal nutrient intake, litter size,

and the location of suckled mammary glands (Kim et al., 2000). The model responds differently to inputs, such as day of lactation, daily nutrient intake, litter size, and the location of the suckled mammary gland. It is used to predict body composition and maternal weight changes during lactation, mammary gland growth during lactation, and growth of nursing pigs.

Our model predicts that when protein intake is decreased, sows will mobilize an increased amount of nutrients from the carcass and internal organs to minimize an effect on milk production (Kim, 1999). This results in small changes in ADG of nursing pigs. The NRC model (NRC, 1998), however, predicts that sows produce lower milk as protein intake decreases, resulting in a significant decrease in ADG of nursing pigs and an increase in maternal protein and fat (Table 3).

The present model can be applied in a practical situation to set a feeding plan for sows during lactation based on the litter growth and the sow body condition from the prediction at targeting weaning day in response to litter size. The present model can also be used to set a nutrient requirement in lactating sows with a concept on balancing optimal body condition of the sow with the maximal mammary gland growth and litter growth. The model will be further developed to use in the estimation of requirements.

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Table 1. Apparent ileal digestibility of some amino acids in major feed ingredients for the lactating sows[1]

	Corn	Barley	Wheat	Soybean meal	Canola meal	Meat and bone meal
n[2]	6	6	6	5	5	5
Crude protein	57.0 ± 2.9	60.2 ± 2.9	80.5 ± 2.9	71.7 ± 3.2	71.7 ± 3.2	61.3 ± 3.2
Lysine	60.5 ± 1.8	72.3 ± 1.8	79.4 ± 1.8	85.6 ± 2.0	85.6 ± 2.0	79.3 ± 2.0
Threonine	62.1 ± 2.5	60.3 ± 2.5	76.7 ± 2.5	76.3 ± 2.7	76.3 ± 2.7	70.2 ± 2.7
Tryptophan	76.9 ± 2.3	76.0 ± 2.3	86.1 ± 2.3	86.4 ± 2.5	86.4 ± 2.5	82.1 ± 2.5
Methionine	79.7 ± 1.4	78.1 ± 1.4	87.4 ± 1.4	82.5 ± 1.5	82.5 ± 1.5	84.0 ± 1.5

[1] Data were adapted from Stein et al. (1999)
[2] Number of sows

Table 2. Amount of amino acids taken up by mammary glands, retained in mammary glands, and accumulated in mammary glands during lactation in the sows

	Amino acids		
	Uptake[1] (g/day)	Retention[1] (g/day)	Tissue growth[2] (g/day)
Lysine	23.5 ± 6.0	1.4 ± 1.1	1.13
Threonine	16.0 ± 4.0	3.5 ± 1.1	0.63
Tryptophan	9.0 ± 1.0	-	0.16
Methionine	7.0 ± 1.5	1.4 ± 0.2	0.31
Leucine	37.0 ± 8.0	10.6 ± 2.4	1.29
Arginine	32.0 ± 7.0	16.5 ± 2.9	0.85
Valine	22.0 ± 4.0	5.5 ± 1.3	0.88
Isoleucine	18.0 ± 4.0	6.3 ± 1.1	0.64
Phenylalanine	15.5 ± 3.0	4.3 ± 1.2	0.70

[1] Data were adapted and modified from Trottier et al. (1997)
[2] Data were adapted and modified from Kim et al. (1999a)

Table 3. Comparison of outputs estimated from the Illinois model (Kim, 1999) and NRC model (NRC, 1998)

Inputs	Daily ME intake, Mcal	16.9	16.9	16.9
	Daily protein intake, g	906	850	790
	Litter size, pigs	10	10	10
Outputs				
Kim, 1999	Average daily gain, g/pig	142.7	141.1	139.1
	Maternal protein gain, g	-174.3	-344.7	445.1
	Maternal fat gain, g	-839.2	-620.4	-243.3
NRC, 1998	Average daily gain, g/pig	200	175	150
	Maternal protein gain, g	-900	1950	4800
	Maternal fat gain, g	-1530	2640	6810

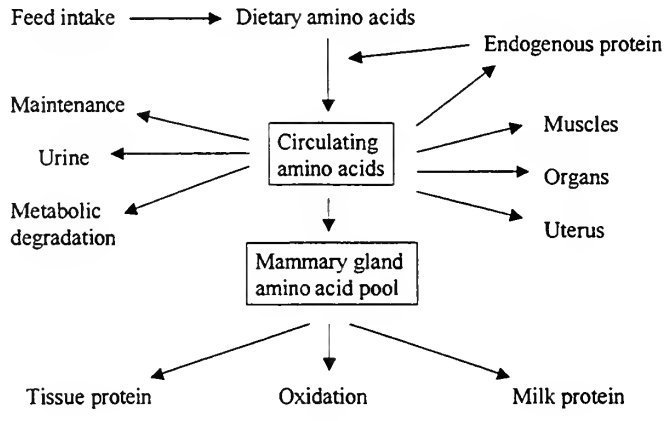

Figure 1. Amino acid flow in lactating sows

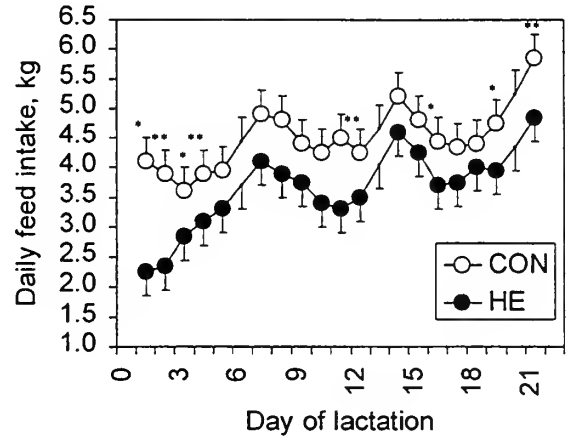

Figure 2. Daily feed intake during lactation in primiparous sows fed a control or a HE diet during gestation. * P < .1 and ** P < .05 (Trottier, 1996)

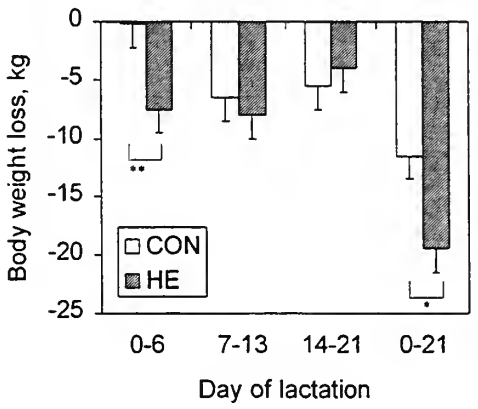

Figure 3. Weekly and total body weight loss during lactation in primiparous sows fed a control (empty bars) or a HE diet (hatched bars) during gestation.
* P < .1 and ** P < .05 (Trottier, 1996)

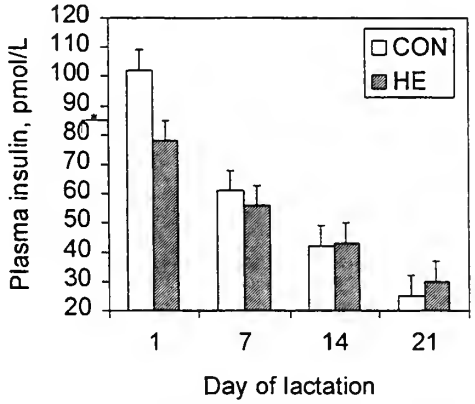

Figure 4. Plasma insulin concentration on d 1, 7, 14, and 21 of lactation in primiparous sows fed a control (empty bars) or a HE diet (hatched bars) during gestation. * P < .1 (Trottier, 1996)

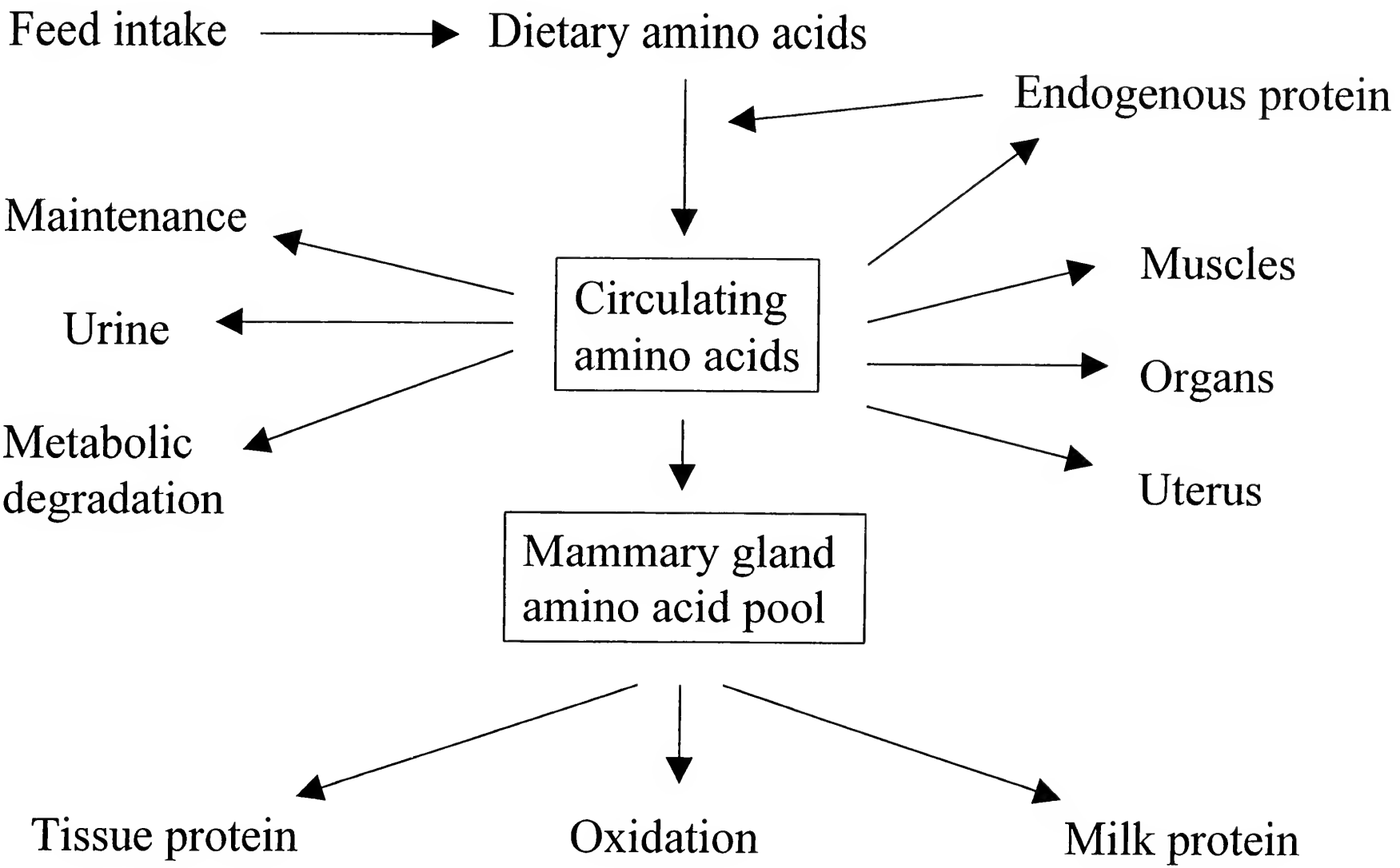

Figure 1. Amino acid flow in lactating sows

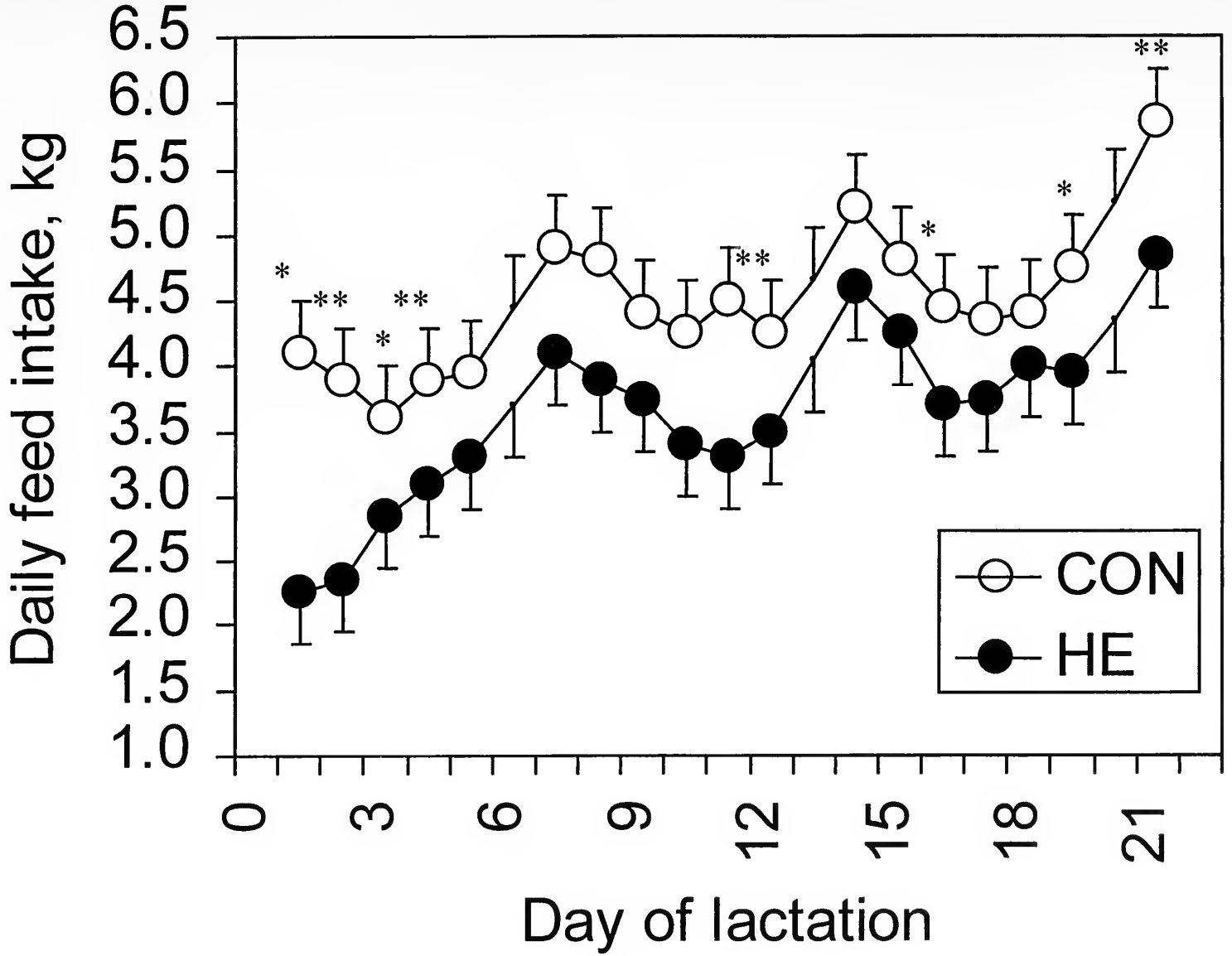

Figure 2. Daily feed intake during lactation in primiparous sows fed a control or a HE diet during gestation. * $P < .1$ and ** $P < .05$ (Trottier, 1996)

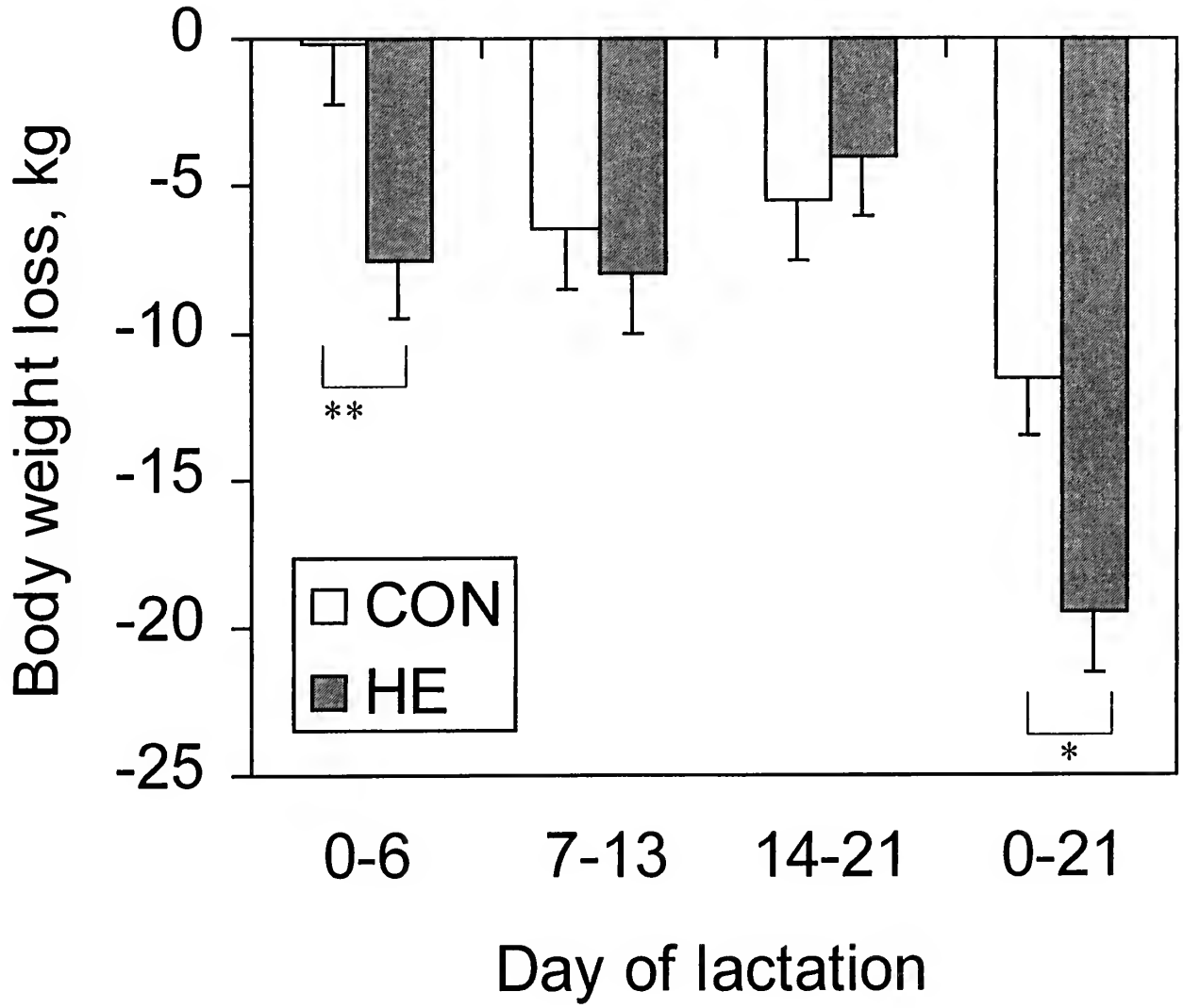

Figure 3. Weekly and total body weight loss during lactation in primiparous sows fed a control (empty bars) or a HE diet (hatched bars) during gestation.
* P < .1 and ** P < .05 (Trottier, 1996)

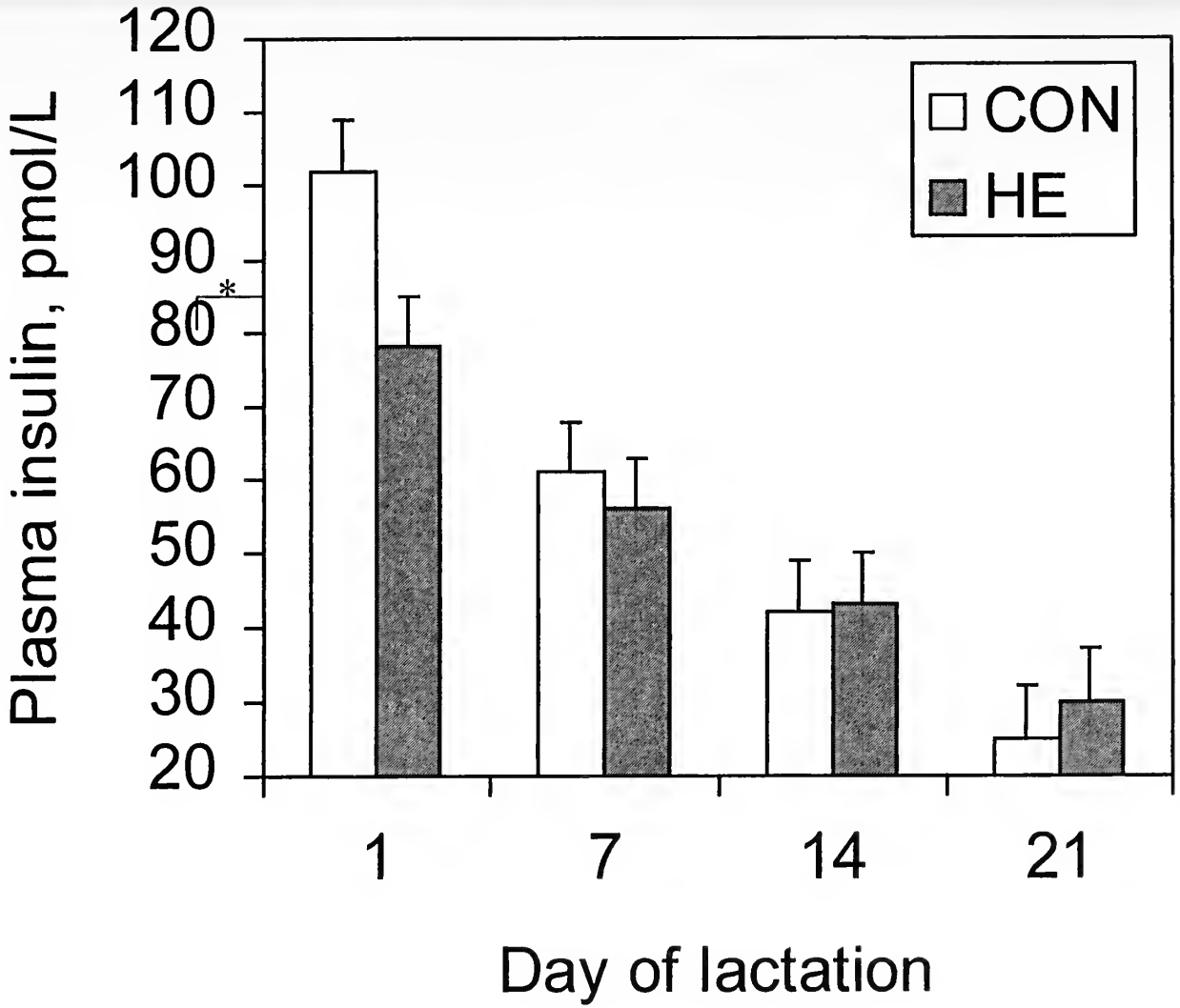

Figure 4. Plasma insulin concentration on d 1, 7, 14, and 21 of lactation in primiparous sows fed a control (empty bars) or a HE diet (hatched bars) during gestation. * P < .1 (Trottier, 1996)

Mammary Gland Growth during Lactation and Its Impact on the Growth of Nursing Pigs

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The sow's mammary gland is the sole organ for providing nutrients for nursing pigs. Understanding the biology of the sow's mammary gland is critical for maximizing pig growth rate during lactation. It has been demonstrated that pre-weaning growth of pigs affects post-weaning growth (McConnell et al., 1987; Mahan and Lepine, 1991; Mahan, 1993). Thus, characterizing growth of mammary gland during lactation and understanding its relationship to the growth of nursing pigs may be important for improving pre-weaning growth of pigs.

Substantial growth and develop of porcine mammary glands occurs during gestation (Hacker and Hill, 1972; Kensinger et al., 1982). However, the extent mammary gland growth during lactation and the importance of that growth relative to the nursing pig had not been demonstrated previously.

Mammary Glands Continue to Grow During Lactation

Fifteen primiparous sows were used to characterize mammary gland growth during lactation (Kim et al., 1999). Sows were killed on d 5, 10, 14, 21, and 28 of lactation and mammary glands were dissected into individual glands. Only mammary glands which were known to be nursed by pigs during lactation were used for chemical analysis.

The wet weight of mammary glands increased 55% from d 5 to d 21 of lactation (Figure 1). The DNA content (mg), which is an indicator of the number of cells in mammary glands, doubled from d 5 to 21 of lactation (Figure 2).

Growth of Nursing Pigs is Strongly Related to the Nursed Mammary Gland

We observed 33 litters from primiparous sows to get information about teat ownership during lactation. This allowed us to know which pig nursed which gland. All sows were killed on d 21 of lactation and mammary glands were dissected into individual glands. Each gland was used for chemical analysis.

Larger glands had greater amounts of DNA ($r = .66$, $P = .0001$), and therefore more cells, than

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smaller glands. Thus, larger glands contained greater amounts of functional tissue than smaller glands which is directly related to milk production. Pigs nursing large glands had a greater growth rate ($r = .67$, $P = .0001$) than pigs nursing smaller glands. The correlation (r) between DNA content (mg) in the glands and growth rate of pigs was .54 ($P = .0001$).

This study demonstrates that porcine mammary glands undergo significant growth during lactation and that this growth of mammary gland is significantly related to the increased capacity for milk production as indicated by the increased mammary wet weight and DNA content. The growth of the mammary glands was related to the increased growth rate of nursing pigs.

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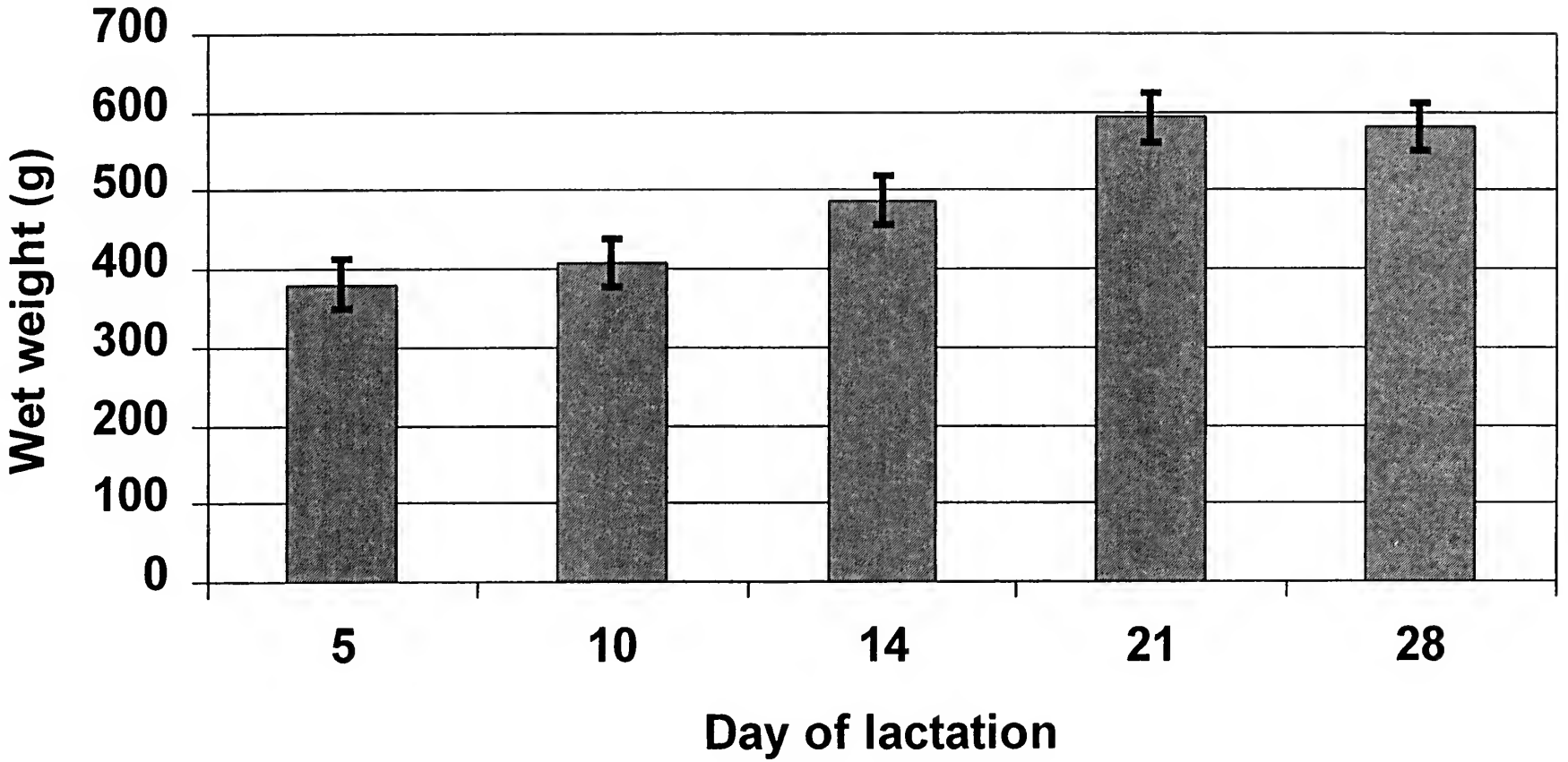

Figure 1. Wet weight of lactating mammary glands at different days of lactation during 28-d period.

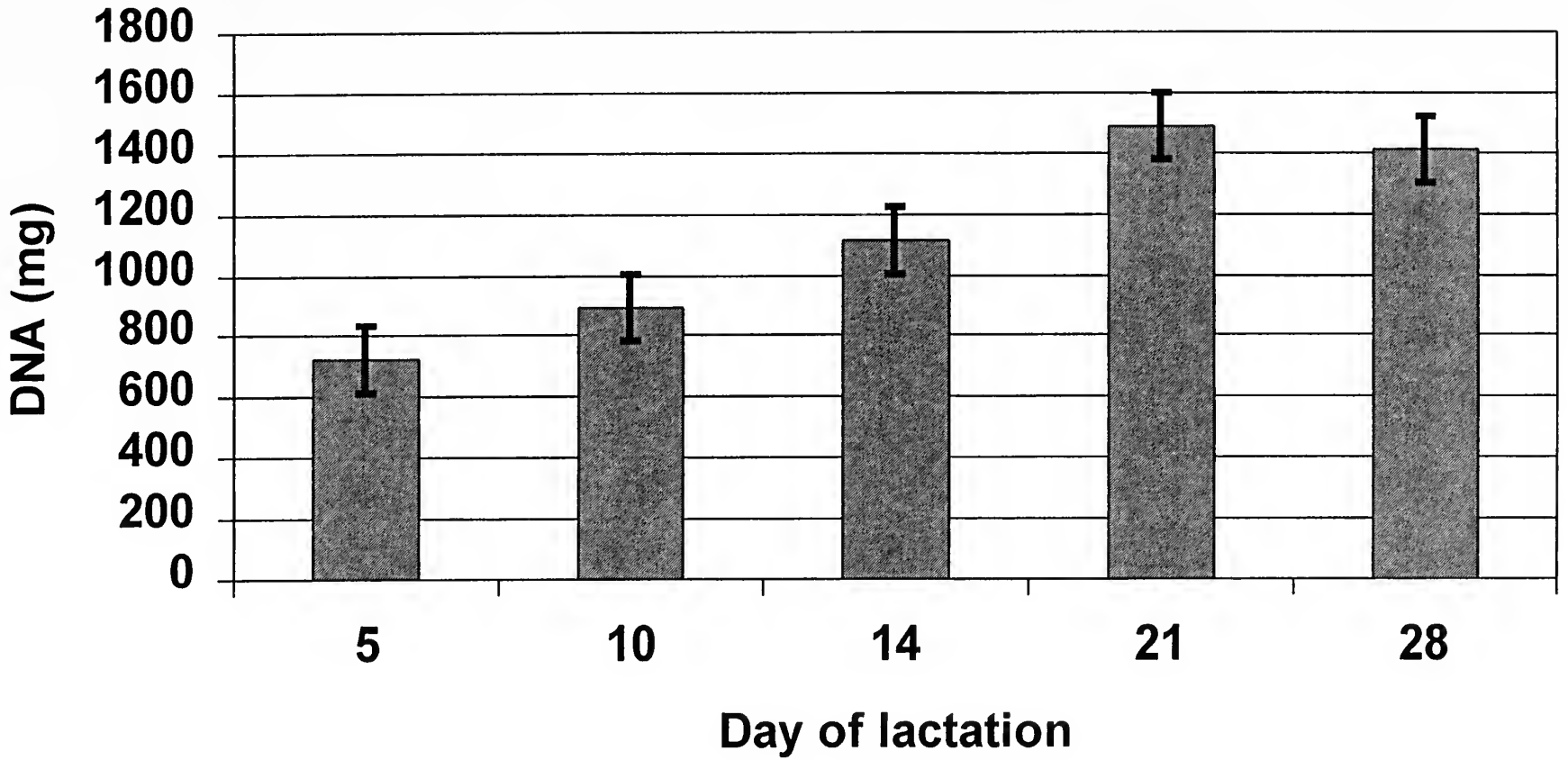

Figure 2. DNA content in lactating mammary glands at different days of lactation during 28-d period.

Growth of Nursing Pigs and Characteristics of Nursed Mammary Gland According to the Anatomical Location

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Within hours after birth, pigs start to nurse the sow and teat order is quickly established (McBride, 1963; Wyeth and McBride, 1964; de Pasille et al., 1988). Sight, smell, and recognition of neighbors are factors which influence the identification of teats by new born pigs (McBride, 1963). Teat order helps to reduce the fighting frequency among littermates (Hartsock and Graves, 1976) and it is reported that teat order at the middle position is more slowly established (Fraser, 1975). Teat order is usually relatively stable (Fraser, 1975). It is believed that heavier or dominant pigs usually nurse anterior mammary glands, which generally leaves the small or subordinate pigs to nurse the posterior glands (English et al., 1977). The variation in weight at weaning is primarily believed to be a result of differences in milk production by each mammary gland (Fraser and Jones, 1975; Fraser et al., 1979). Weight variations within a litter can result in greater complexity of management of pig movement through consecutive phases of production by delaying weaning or marketing.

It has been suggested that the anterior mammary glands may be larger, produce more milk, or provide a more comfortable nursing position for pigs (Donald, 1937; Gill and Thomson, 1956), whereas others have suggested that there is no difference in milk production among glands (Hartman et al., 1962; Pond et al., 1962). The true advantages for pigs nursing anterior mammary glands remains uncertain. The objectives of this study were to show how growth of nursing pigs is influenced by the characteristics of nursed mammary glands, and how this effect is may vary according to anatomical location of the glands.

Data from two different experiments are summarized. Thirteen primiparous sows and litters (litter size was 8.7 ± 1.5) and twenty primiparous sows and litters (litter size was 8.9 ± 1.4) were used for Experiments 1 and 2, respectively. Teat order for each litter was observed to know which mammary glands were nursed by which pigs. The weights of individual pigs were recorded at birth and weekly until weaning. Mammary glands were collected at slaughter on d 21 of lactation and trimmed of skin and the extraneous fat pad. Individual glands were separated, weighed, and ground for measurement of DNA content. Another eight primiparous sows were killed within 12 h after farrowing to establish the characteristics of mammary glands at farrowing.

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Pigs Prefer Anterior and Middle Mammary Glands

More than 60% of the first four pairs of mammary glands were nursed by pigs while pigs nursed less than 40% of the seventh and eighth pairs of mammary glands (Figure 1). This distribution of gland use is similar to previous reports (English et al., 1977). The advantages of nursing anterior or middle mammary glands may be greater milk yield, better space availability, greater teat length, greater opportunity to obtain nutrients from the blood circulation, and other unknown causes. Demonstrating the differences in size and composition of the mammary glands according to their anatomical location may provide clues to why the gland use is not uniform.

Mammary Gland Selection is Not Affected by Birth Weight of Nursing Pigs

From this study, there was no relationship between birth weights of pigs and the anatomical location of nursed mammary glands (Table 1). This finding agrees with previous work by Hemsworth et al. (1976), but is inconsistent with a study by Scheel et al. (1977) who showed that pigs with greater birth weight had specific preference for anterior glands ($r = .49$, $P < .01$). The different results of these studies might indicate that modern sows have a greater number of well-developed mammary glands available at front and middle than the sows in the past.

Growth of Nursing Pigs Was Affected by Anatomical Location of the Nursed Mammary Gland

By d 21 of lactation, the average daily gain (ADG) of pigs was different according to the anatomical location of nursed mammary glands (Table 1). The average daily gain of the pigs which nursed the first five pairs of glands was 29% greater than ADG of pigs which nursed posterior glands. Anterior and middle glands may provide greater amounts of nutrients to nursing pigs than posterior glands.

Characteristics of Mammary Glands Vary According to Their Anatomical Location

At farrowing (within 12 h of farrowing), the middle mammary glands (fourth and fifth pairs) were greater in size and contents of protein and DNA than anterior (first to third pairs) and posterior (sixth to eighth pairs) glands (Table 2). However, after a 21-d lactation, the size and DNA contents of anterior glands were as great as middle glands, while posterior glands were smaller (Table 2). The rate of growth of individual mammary glands during lactation may not be the same according to their anatomical location during lactation. There should be an advantage for pigs nursing the anterior and middle glands, compared with posterior glands.

Conclusions

Pigs which nursed anterior and middle glands had greater ADG than pigs which nursed posterior glands. There were no significant weight differences among pigs which nursed anterior and middle glands. Pig weight variation within a litter may be minimized when pigs nurse ten mammary glands at anterior or middle location. The availability of 10 high producing glands per sow coincides with the average litter size of 10 in the U.S.

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Table 1. Birth weight and average daily gain of nursing pigs by anatomical location of the nursed mammary glands[a]

Anatomical location of the glands[b]	Exp. 1			Exp. 2		
	n	Birth weight	ADG[c]	n	Birth weight	ADG[c]
		(kg)	(g)		(kg)	(g)
1	20	1.48±.03[de]	161.1±6.1[d]	32	1.54±.04	159.9±6.4[de]
2	20	1.46±.03[de]	161.2±6.0[d]	36	1.52±.04	159.6±6.1[de]
3	15	1.53±.03[d]	153.3±6.1[de]	35	1.56±.04	164.0±4.2[d]
4	19	1.47±.03[de]	152.8±6.3[de]	26	1.53±.04	157.2±7.2[de]
5	10	1.52±.04[de]	163.6±7.3[d]	19	1.58±.05	159.3±8.4[de]
6	14	1.42±.04[ef]	135.8±8.0[ef]	20	1.56±.05	145.2±8.2[def]
7	7	1.32±.06[f]	127.0±11.0[f]	9	1.48±.07	124.3±12.3[f]
8	1	1.37±.13[def]	116.5±21.6[ef]	1	1.77±.22	90.2±36.8[ef]

[a] Least-squares mean ± standard error of the mean.

[b] 1 = anterior.

[c] Average daily gain of nursing pigs.

[d,e,f] Within a column, means lacking a common superscript letter differ ($P < .05$).

Table 2. Weights and composition of mammary glands according to anatomical location[a]

	Anatomical location of the glands (1 = anterior)							
	1	2	3	4	5	6	7	8
d 0 of lactation (within 12 h after farrowing)								
Number of nursed glands	16	16	16	16	16	16	7	1
Wet weight (g)	327±31^{j}	436±31^{hi}	452±31^{gh}	530±31^{fg}	542±31^{f}	359±31^{ij}	322±48^{j}	291±126^{fghij}
DNA (mg)	605±56^{ij}	829±56^{h}	911±56^{gh}	1084±56^{f}	1028±56^{fg}	632±56^{i}	428±84^{j}	409±222^{hij}
d 21 of lactation from Exp. 1								
Number of nursed glands	20	20	15	19	10	14	7	1
Wet weight (g)	452±38^{g}	578±38^{f}	581±44^{f}	524±39^{fg}	515±54^{fg}	489±46^{fg}	436±65^{fg}	457±172^{fg}
DNA (mg)	1088±139^{fg}	1379±139^{f}	1487±161^{f}	1205±142^{fg}	1431±196^{f}	1125±166^{fg}	787±234^{g}	604±619^{fg}
d 21 of lactation from Exp. 2								
Number of nursed glands	32	36	35	26	19	20	9	1
Wet weight (g)	465±20^{fg}	516±19^{f}	511±19^{f}	500±22^{f}	462±26^{fg}	458±25^{fg}	387±38^{g}	341±112^{fg}
DNA (mg)	934±44^{gh}	1053±41^{f}	1047±42^{fg}	1084±49^{f}	1020±57^{fgh}	893±56^{h}	660±83^{i}	668±250^{fghi}

[a] Values are least-squares mean ± standard error of the mean.

^{f,g,h,i,j} Within a row, means lacking a common superscript letter differ ($P < .05$).

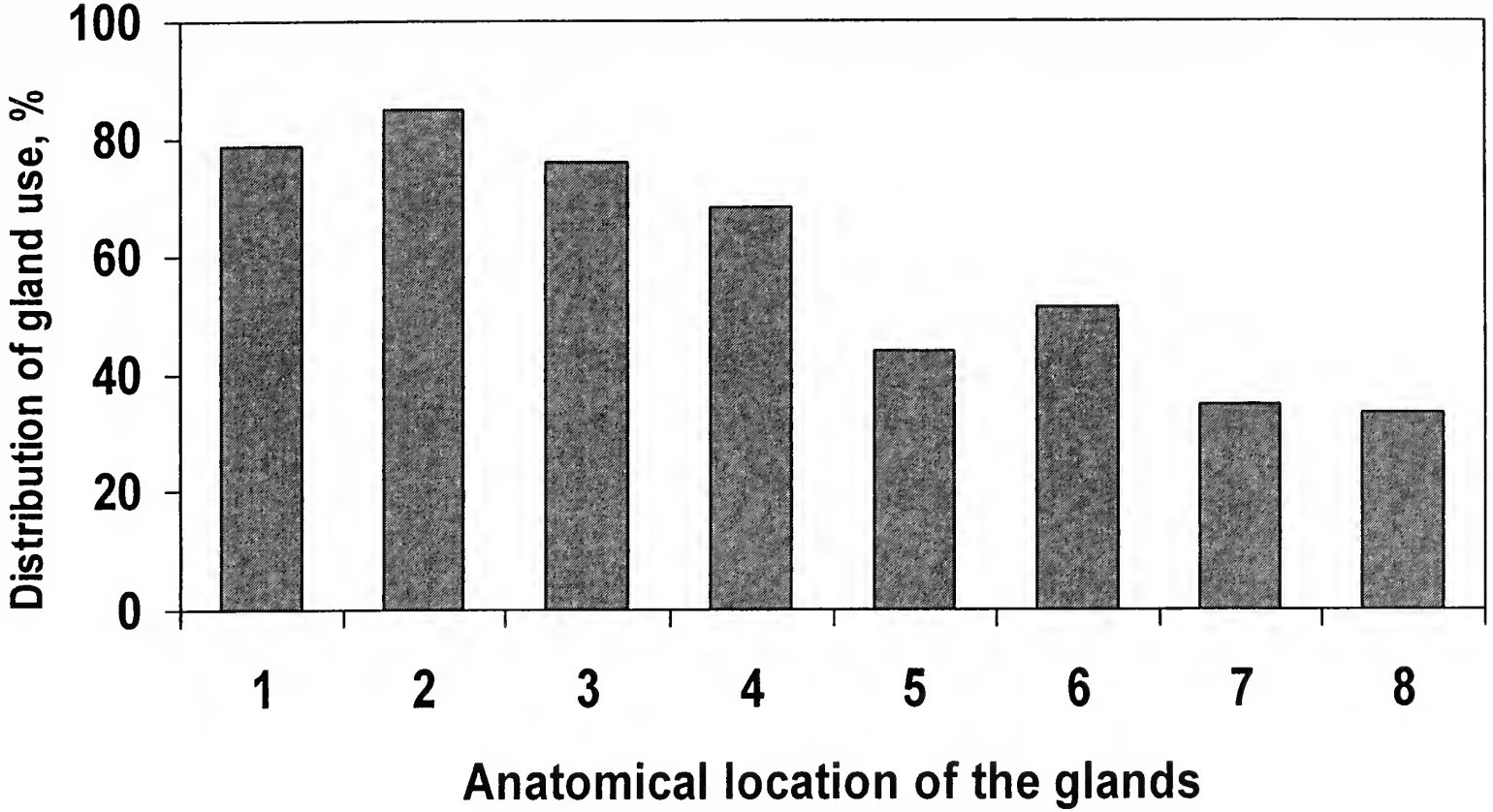

Figure 1. Distribution of gland use by the nursing pigs according to the anatomical location of the nursed mammary glands (1 = anterior) from Exp. 1 and Exp. 2.

Dietary Nutrient Affects Mammary Gland Growth in Lactating Sows

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There are many factors that can potentially affect mammary gland growth. Estrogen and progesterone are known as essential hormonal factors for the growth of mammary glands during lactation. Insulin, cortisol, thyroid hormone, and prolactin also support mammary gland growth. Nutrient intake may also affect the growth of mammary glands. During gestation, dietary protein level does not affect mammary gland growth of gilts (Weldon et al., 1991; Kusina et al., 1999). However, excessive energy intake during gestation reduces mammary gland growth of gilts (Weldon et al., 1991).

The lactation period is a challenge for the sow. Milk production results in a high demand for nutrients by the sow and sows will mobilize their body tissues when dietary nutrients do not meet the demands for milk production. However, the mammary glands are also growing during lactation (Kim et al., 1999a), even though sows are in a catabolic state. It may be expected that level of dietary nutrient intake during lactation may impact mammary gland growth, although this relationship has not been demonstrated previously.

Protein and Energy Intake Affect Mammary Gland Growth During Lactation

Sixty-one primiparous sows were fed four different diets containing two levels of protein and two levels of energy (Kim et al., 1999b). Teat order was observed from each litter one day prior to killing of sows to know which glands were nursed during lactation. Sows were killed at times up to 30 d of lactation. Mammary glands were dissected into individual glands and only glands known to have been nursed by pigs during lactation were used for these analysis. Wet weight of individual glands was recorded.

Both protein intake and energy intake of lactating sows affected the growth of the mammary gland during lactation. Growth of mammary glands was stimulated by increased dietary protein and energy during lactation. We generated three-dimensional response surface graphs to show the effects of protein and energy on mammary gland growth as lactation progressed (Figure 1). The x-axis represents total or accumulative energy intake of the sow as lactation progresses, the y-axis represents total protein intake of the sow, and the z-axis represents response variables, such

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as wet weight and DNA content. The highest point of the graph indicates the combination of dietary protein and energy when mammary gland growth was maximized. Thus, the day of lactation and dietary protein and energy levels when sows had maximum mammary gland growth during lactation can be estimated. Mammary gland growth of lactating sows was maximized when sows consumed daily 16.9 Mcal ME and 55 g lysine (990 g crude protein). Maximal mammary gland size occurred between d 25 and 28 of lactation. Feeding sows adequately for maximal mammary gland growth should be emphasized for improving weaning weight of pigs. Both protein intake and energy intake during lactation should be considered when accounting for mammary growth.

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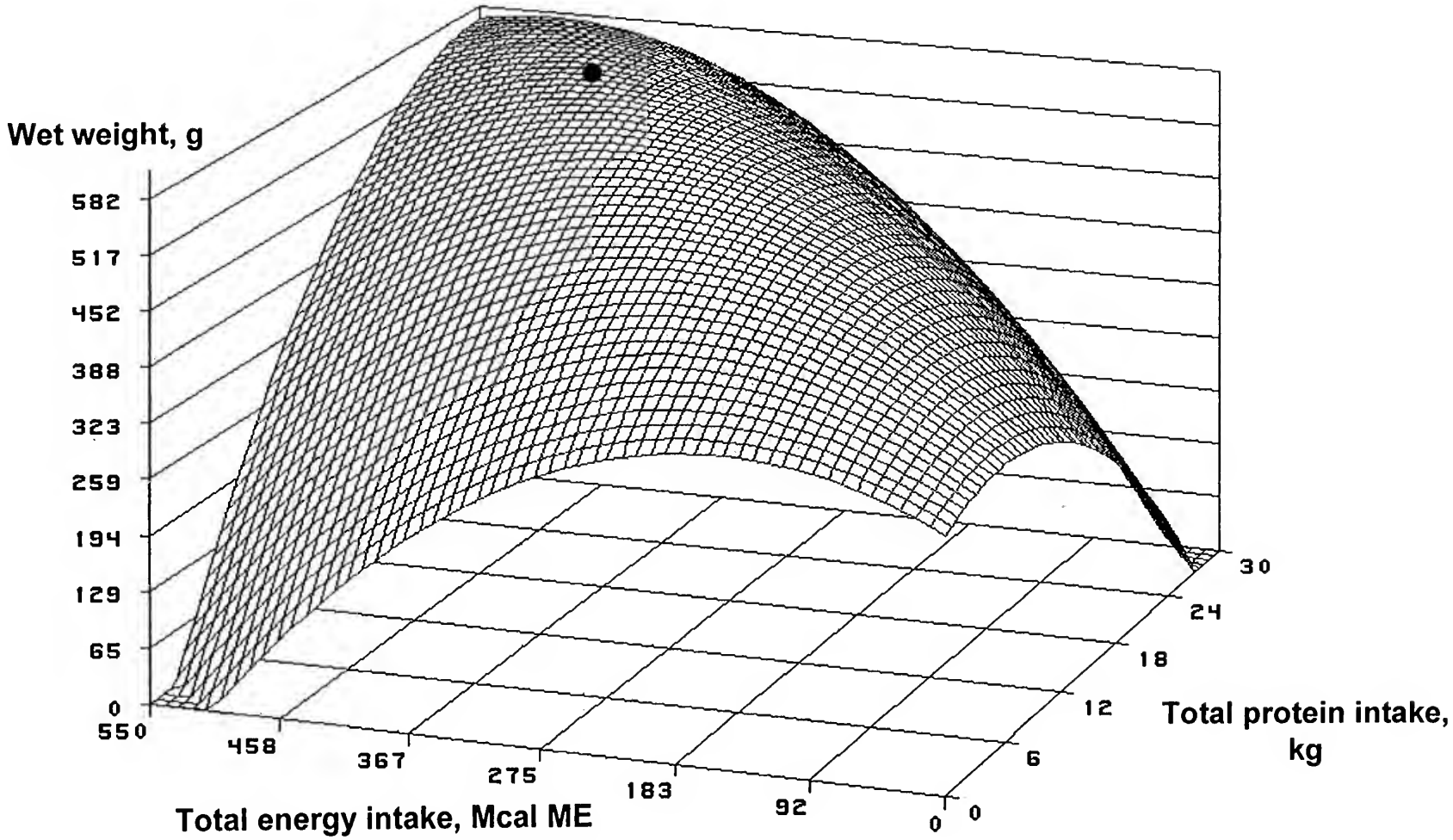

Figure 1. Response surface plot of the wet weight of nursed mammary glands from sows fed different levels of energy and protein. ● = maximal point of the response surface.

Litter Size Affects Mammary Gland Growth in Lactating Sows

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Sows with larger litters produce greater amounts of milk than sows with smaller litters. Sows with 12 pigs produced more than 50% more milk than sows with 6 pigs (King et al., 1989; King, 1991). This may result from an increased number of active mammary secretory cells, a crucial component of milk production as litter size increases (Tucker, 1966; Knight and Peaker, 1984; Knight et al., 1984). Elsley (1971) recognized that there is a relationship between litter size and milk output. The demand for milk by the young parallels increases in litter size and the sow responds, within biological constraints, by producing more milk which results in increased litter weight gain (Auldist et al., 1998). Increased mammary growth during lactation seems to be stimulated by an increased demand for milk production. Tucker (1964) demonstrated that litter size affected mammary growth in lactating rats. Weight of total mammary tissue and mammary DNA increased as the number of nursing pups increased. Our hypothesis is that the growth of the porcine mammary gland during lactation will respond to litter size differences. In this paper, we show that the litter size affects mammary gland growth during lactation and we discuss its implication on nutritional aspect of sows.

Twenty-eight primiparous sows were used in this study and each group of four sows had different litter sizes of 6, 7, 8, 9, 10, 11, or 12. Each litter was observed to determine which mammary glands were nursed during lactation. All sows were killed on d 21 of lactation to obtain mammary glands. Mammary glands were dissected into individual glands and only glands know to have been nursed by pigs during lactation were used for chemical analysis.

Sows with Large Litter Size Have Greater Amount of Total Mammary Tissue and Total Mammary Cells

Sows with 12 pigs had 2,098, 227, and 4.4 g more mammary tissue wet weight, protein, and DNA, respectively, than sows with 6 pigs when measured on d 21 of lactation (Kim et al., 1999b). This corresponds to 65%, 63%, and 67% increases for those tissue components, when litter size doubled. Doubling litter size also increased litter weight gain by 96% or 18.1 kg. Mammary glands in sows with larger litters may be more efficient at producing milk because the

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increase in litter weight gain was greater than the increase in mammary components in response to increased litter size.

However, Individual Glands Are Smaller in Sows with Larger Litter

Within individual nursed mammary glands, the wet and dry weights, and the amounts of DFFT, protein, DNA, fat, and ash decreased as litter size increased. These decreases in mammary tissue mass correspond with decreases in the weight gain of individual pigs as litter size increases.

Our results are consistent with previous studies. Total milk production increases as litter size increases, but milk yield per nursing pig decreases (Elsley, 1971; King et al., 1989; Auldist et al., 1998). Auldist et al. (1998) showed that total litter weight gain increases as litter size increases, but there is decreased weight gain per nursing pig.

Impact on Nutrition of Lactating Sows

As litter size increases, sows need more nutrients to support increased milk production. Sows also need to provide nutrients for supporting the growth of the increased number of lactating mammary glands as litter size increases. For example, when it is assumed that the lysine content in mammary tissue protein is 7.5% (Kim et al., 1999a), the amount of lysine accretion in mammary tissue as litter size changes from 6 to 12 pigs is 17 g during a 21-d lactation, or about .8 g per day. Kim et al. (1999a) showed that 14% of total essential amino acids taken up by the mammary gland, but not excreted as milk protein, were accumulated in mammary protein. Thus, 5.71 g lysine per day was used for the additional growth of mammary glands as litter size increased 6 to 12. It can be estimated that sows need an additional .95 g lysine per day for each pig added to the litter from 6 up to 12 to meet the increased requirements for mammary tissue growth. When considering nutrient needs for sows with large litters, nutrients for additional mammary gland growth as well as increased milk production should be considered.

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Ileal Digestibilities of Amino Acids in Growing Pigs fed Soybean Meal and Corn Starch-Based Diets Supplemented with Fiber-Degrading Enzymes and Steeping

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Introduction

The digestibility of amino acids and other nutrients by the pig is an important measure of efficiency when evaluating swine diets or ingredients used in swine diets. Any method of feeding or use of a feed additive that significantly increases the ileal digestibility of amino acids could be used to increase the efficiency of amino acid use by the pig, and decrease waste and nutrient losses from swine production.

Feed enzymes have been used in an attempt to increase nutrient digestibility in swine (Bedford and Schulze, 1998). Much of the research with fiber-degrading enzymes has been conducted with beta-glucanase (Baas and Thacker, 1996; Li et al., 1996; Baidoo et al., 1998), which is commonly used in diets that mainly consist of barley or wheat. Research conducted with cellulase and hemicellulase is less common, and the influence of these two enzymes used in combination has not been evaluated. Soybean meal contains 3.39% cellulose and 3.48% hemicellulose on a dry matter basis (Gabert, 1994). Fibrous components of the diet, especially cellulose, have been shown to decrease amino acid digestibility in the pig (Li et al., 1994; Lien, 1995). Thus, cellulase and hemicellulase may improve the ileal digestibility of amino acids in soybean meal.

Feed steeping (soaking meals in water prior to feeding) has been evaluated, and has been shown to increase weight gain and feed efficiency, as well as the efficacy of supplemental phytase and its effects on phosphorus retention, in a corn-soybean meal diet for growing pigs (Liu et al., 1997). Skoglund et al. (1997) also reported improvements in phosphorus utilization when a barley-rapeseed cake-pea diet was steeped. However, Nasi and Helander (1994), while conducting research with phytase, reported that soaking did not affect the digestibility or retention of any nutrient in a barley-soybean meal diet.

The purpose of the present study was to evaluate the effect of cellulase and hemicellulase, feed steeping for 12 h prior to feeding, and the combination of both of these treatments, on ileal amino acid digestibilities in growing pigs fed semipurified diets with soybean meal as the sole protein source.

Materials and Methods

Animals and treatments. Eight barrows (Line 326 sire x C22 dams; PIC, Franklin, KY) with an average initial body weight of 69.9 kg were surgically fitted with a simple-T cannula at the distal ileum using procedures adapted from Sauer et al. (1983). The barrows were housed in individual metabolism crates in a temperature-controlled barn, and were used for two experimental periods. Two experimental diets with (Diet B) and without (Diet A) fiber-degrading enzymes, formulated to contain 17% crude protein and .5% chromic oxide, were used (Table 1). The enzyme diet (B) contained 30,080 units of cellulase/kg diet and 531 units of hemicellulase/kg diet. The barrows were fed one of four experimental treatments – control diet (A); control diet plus steeping for 12 h prior to feeding; enzyme diet (B); and the enzyme diet plus steeping for 12 h prior to feeding. Water was added to the steeped meals on a 1:1 weight basis, and was available to all pigs from a low-pressure drinking nipple ad libitum. Each period consisted of a 5 d adaptation phase and 2 d collection phase. The pigs were fed 1.76 kg/d in the first period, and 1.84 kg/d in the second period. Feeding occurred at 0800 and 2000 h. Ileal digesta were collected for 12 h intervals, from 0800 to 2000 h, over the two days. Digesta was frozen at –10°C after collection, and was pooled within barrow during the first period. During the second period, digesta was pooled within barrow and day of collection to determine the effect of day on digestibility calculations.

Chemical and statistical analysis. Samples of diets, freeze-dried digesta and soybean meal were ground with liquid nitrogen in a coffee mill. For dry matter determinations, empty 50 mL glass beakers that had been in a 105°C oven overnight were weighed immediately after removal from the oven. Samples of diets and digesta were then weighed into the beakers, and the beakers plus sample were placed into the oven overnight. The next day each beaker and sample were weighed, and dry matter was calculated. The same samples were then ashed in a muffle furnace for 16 h at 450°C. Organic matter was determined by the difference between ash percentage and 100. Then, the samples were used for chromic oxide determination according to Fenton and Fenton (1979). Samples of diets and digesta were analyzed for crude protein according to the AOAC (1995). Amino acid levels were determined in the diets, digesta, soybean meal, cellulase and hemicellulase using pre-column derivatization with phenyl isothiocyanate. Samples were weighed into screw-capped test tubes, and 6 M HCl was added to the tubes. The tubes were purged with nitrogen and were hydrolyzed in an oven for 24 h at 110°C. Amino acids were determined in our laboratory with procedures adapted from Bidlingmeyer et al. (1984). Digesta, cellulase and hemicellulase were analyzed in duplicate, soybean meal in triplicate, and five samples of each diet were analyzed. Correction factors determined in our laboratory were used to more accurately reflect amino acid concentrations in both diets and digesta.

In the second period, day was found to have no influence ($P > .05$) on apparent ileal digestibility. Therefore, digestibility coefficients for day 1 and day 2 were not pooled, and thus, six replicates per treatment were obtained. Also, the use of correction factors did not affect ($P > .05$) digestibility determinations when compared to those determined without correction factors. The apparent ileal digestibility values of the experiment treatments were subjected to statistical analysis as a two-period crossover design with six replicates per treatment using PROC GLM of SAS (1990). Means of dietary treatments and experimental periods were compared using Fisher's protected least significant difference procedure (pdiff procedure, SAS; Milliken and Johnson, 1984).

Results and Discussion

The pigs remained healthy and consumed their daily meals throughout the experiment. The apparent ileal digestibilities of dry matter, crude protein, organic matter and amino acids are presented in Table 2. Digestibility was not affected ($P > .05$) by any of the treatments. There were small numerical increases in digestibility with the use of enzymes, as well as with the use of steeping. The use of fiber-degrading enzymes in conjunction with steeping numerically reduced the digestibility of some amino acids. To our knowledge, this is the first study to investigate the influence of a combination of fiber-degrading enzymes on the ileal digestibility of amino acids. Our findings with regard to steeping are in agreement with those of Nasi and Helander (1994).

Implications

Fiber-degrading enzymes and steeping did not affect ileal amino acid digestibility in growing pigs. Further research with pigs at different ages and physiological states, as well as different diets, should be conducted to further evaluate the efficacy of fiber-degrading enzymes and steeping.

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Table 1. Formulation (%) of the experimental diets (as-fed basis)

	Diet	
Ingredient	*A*	*B*
Corn starch[a]	38.95	35.68
Soybean meal	34.66	34.66
Sucrose[b]	20.00	20.00
Corn oil	2.00	2.00
Dicalcium phosphate	3.00	3.00
Calcium carbonate	0.45	0.45
Vitamin premix[c]	0.10	0.10
Trace mineral premix[d]	0.34	0.34
Cellulase[e]	0.00	0.32
Hemicellulase[f]	0.00	2.95
Chromic oxide	0.50	0.50

[a]Clinton® Brand, Archer Daniels Midland Company, Effingham, IL

[b]Domino® Sugar, Tate & Lyle North American Sugars, Inc., New York, NY

[c]Provided the following (per kg of diet): choline, 121 mg; niacin, 16.5 mg; pantothenic acid, 12.1 mg; riboflavin, 4.40 mg; vitamin B_{12}, .020 mg; vitamin E, 44.1 IU; vitamin A, 3310 IU; vitamin D, 331 IU; vitamin K, 2.21 mg. Supplied by Archer Daniels Midland Company, Effingham, IL.

[d]Provided the following (per kg of diet): sodium, 2.80 g; chloride, 2.80 g; copper, 7.79 mg; iodine, .34 mg; iron, 87.4 mg; manganese, 19.4 mg; selenium, 0.29 mg; zinc, 97.2 mg. Supplied by Archer Daniels Midland Company, Effingham, IL.

[e]Cellulase, Sigma Chemical Company, cat. # C-0901, provided 30,080 enzyme units per kg diet.

[f]Hemicellulase, Sigma Chemical Company, cat. # H-2125, provided 531 enzyme units per kg diet.

Table 2. Apparent ileal digestibilities (%) of dry matter, organic matter, crude protein and amino acids in the experimental treatments

	Treatment[a]				
Item	*A*	*A plus steeping*	*B*	*B plus steeping*	*SEM*[b]
Dry matter	81.6	80.9	83.4	81.7	0.90
Organic matter	85.2	84.8	87.1	85.4	0.80
Crude protein	81.8	81.7	84.3	82.8	1.25
Amino acids[c]					
Indispensable					
Arginine	90.3	91.2	91.6	91.1	1.02
Histidine	88.3	92.6	82.6	88.7	2.96
Isoleucine	84.2	85.3	86.3	85.5	1.08
Leucine	82.1	82.6	84.2	82.8	1.25
Lysine	86.1	87.8	87.0	87.9	1.36
Phenylalanine	84.9	85.3	86.0	84.4	1.06
Threonine	78.3	82.1	82.7	83.4	2.24
Valine	79.8	80.6	81.3	79.8	1.32
Dispensable					
Alanine	79.2	81.8	80.2	80.4	1.60
Aspartic acid	79.5	79.7	79.7	77.7	1.46
Glutamic acid	83.3	82.0	83.6	82.3	1.66
Glycine	70.4	70.1	67.0	63.8	4.27
Serine	79.5	80.6	82.3	80.7	2.00
Tyrosine	84.9	85.2	85.0	85.5	1.33
Proline	76.6	81.8	81.1	80.1	3.07

[a]Treatment did not affect ($P > .05$) apparent ileal digestibility.

[b]Standard error of the mean (n=6).

[c]Amino acid digestibility coefficients calculated using correction factors determined in our laboratory. See Materials and Methods.

The Effect of Enzymes, Steeping and Dietary Protein Level on Apparent Fecal Digestibility and Fecal Output in Pigs fed Corn-Soybean Meal Diets.

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Introduction

The digestibility of nutrients by the pig is an important measure of efficiency when evaluating swine diets or ingredients used in swine diets. Any method of feeding or feed additive that significantly increases the digestibility of nutrients could be used to increase the efficiency of, and decrease waste and nutrient losses from, swine production.

Feed enzymes have been used to attempt to increase nutrient digestibility in swine (Bedford and Schulze, 1998). Much of the research with fiber-degrading enzymes has been conducted with beta-glucanase (Baas and Thacker, 1996; Li et al., 1996; Baidoo et al., 1998), which is commonly used in diets that mainly consist of barley or wheat. Research conducted with multiple enzymes in the same diet is less common, and the influence of enzymes used in combination on digestibility of nutrients has not been evaluated. Fibrous components of the diet, especially cellulose, have been shown to decrease amino acid digestibility in the pig (Li et al., 1994; Lien, 1995). Thus, a mixture of mainly fiber-degrading enzymes may improve the digestibility of nutrients in the corn-soybean meal diet.

Feed steeping (soaking meals in water prior to feeding) has been evaluated, and has been shown to increase weight gain and feed efficiency, as well as the efficacy of supplemental phytase and its effects on phosphorus retention, in a corn-soybean meal diet for growing pigs (Liu et al., 1997). Skoglund et al. (1997) also reported improvements in phosphorus utilization when a barley-rapeseed cake-pea diet was steeped. However, Nasi and Helander (1994), while conducting research with phytase, reported that soaking did not affect the digestibility or retention of any nutrient in a barley-soybean meal diet.

The purpose of the present study was to evaluate the effect of six enzymes (cellulase, hemicullulase, amylase, xylanase, alpha-galactosidase and protease), feed steeping for 12 h prior to feeding, and the combination of both of these treatments, on the apparent fecal digestibility of crude protein in, and fecal output of, growing pigs fed corn-soybean meal diets.

Materials and Methods

Animals and treatments. Eight barrows (Line 337 sire x C22 dams; PIC, Franklin, KY) with an average initial body weight of 43 kg were randomly assigned to two 4 x 4 Latin squares. The barrows were housed in individual metabolism crates in a temperature-controlled barn, and were used for four experimental periods. Four experimental diets, formulated to contain either 14% or 18% crude protein and .3% chromic oxide, were used. Two of the diets contained supplemental enzymes (diets B and D), and two of the diets did not contain enzymes (diets A and C; Table 1). The diets with enzymes contained 3,400 units of cellulase/kg diet; 165,000 units of hemicellulase/kg diet; 2,128 units of amylase/kg diet; 21,280 units of protease/kg diet; 1,596 units of xylanase/kg diet; and 1,800 units of alpha-galactosidase/kg diet. The barrows were fed one of four experimental treatments in each square – control diet (A or C); control diet plus steeping for 12 h prior to feeding; enzyme diet (B or D); and the enzyme diet plus steeping for 12 h prior to feeding. Water was added to the steeped meals on a 1.45:1 weight basis, and was available to all pigs from a low-pressure drinking nipple ad libitum. Each period consisted of a 4 d adaptation phase and 5 d collection phase with total fecal collection. The pigs were fed 1.8, 1.92, 2.0, and 2.06 kg/d respectively during periods 1-4. Feeding occurred at 0900 and 2100 h. Feces were pooled within barrow and period, and were then frozen at –10°C.

Chemical and statistical analysis. Samples of diets and 57°C oven-dried feces were ground with liquid nitrogen in a coffee mill. For dry matter determinations, empty 50 mL glass beakers that had been in a 105°C oven overnight were weighed immediately after removal from the oven. Samples of diets and feces were then weighed into the beakers, and the beakers plus sample were placed into the oven overnight. The next day each beaker and sample were weighed, and dry matter was calculated. The same samples were then ashed in a muffle furnace for 16 h at 450°C. Organic matter was determined by the difference between ash percentage and 100. Then, the samples were used for chromic oxide determination according to Fenton and Fenton (1979). Crude protein was determined for diets and feces according to the AOAC (1995).

The apparent fecal digestibility values and fecal output of the experiment treatments were subjected to statistical analysis as Latin square designs with four replicates per treatment using PROC GLM of SAS (1990). Means of dietary treatments and experimental periods within square were compared using the Student-Newman-Keuls multiple range test (Ott, 1993). One-way ANOVA was used to evaluate the effect of protein level (square) on the measured parameters (SAS, 1990).

Results and Discussion

The apparent fecal digestibilities of dry matter, crude protein and organic matter, and fecal output are presented in Table 2. Digestibility was not affected ($P > .05$) by any of the treatments. The use of feed enzymes in conjunction with steeping numerically reduced crude protein digestibility. Fecal output on a wet basis was not affected ($P > .05$) by any of the treatments. However, pigs in the 18% crude protein square fed supplemental enzymes tended to produce less feces ($P = .07$) on a wet basis than all other treatments. Fecal output on a dry matter basis from pigs in the 14% crude protein square was not affected ($P > .05$) by treatment. However, in the 18% crude protein square, pigs fed the enzyme-supplemented diet produced fewer feces ($P <$

.05) on a dry matter basis. Thus, the use of multiple enzymes resulted in fewer fecal solids produced compared to all other treatments in the 18% crude protein square. Apparent protein digestibility and fecal output, both on a dry and wet basis, were higher ($P < .05$; $P = .08$) for pigs fed the 18% crude protein diet compared to those fed the 14% crude protein diet, which were expected (Data not shown). To our knowledge, this is the first study to investigate the influence of a combination of enzymes on nutrient digestibility and fecal output using a corn-soybean meal diet.

Implications

Multiple enzymes and steeping did not affect fecal digestibility in growing pigs fed corn-soybean meal diets with 14% or 18% crude protein. Fecal output was not affected by treatments in the 14% crude protein square, but fecal output on a dry matter basis was lowered by the use of feed enzymes in the 18% crude protein square. Also, pigs fed 18% crude protein diets had higher crude protein digestibility coefficients and fecal output. Thus, feeding growing pigs 18% crude protein diets supplemented with the six enzymes used in this study at a total dietary inclusion level of 1% reduced fecal output. Further research with pigs at different ages and physiological states, as well as different diets, should be conducted to further evaluate the efficacy of feeding multiple enzymes and steeping.

Acknowledgments

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Table 1. Formulation (%) of the experimental diets (as-fed basis)

	Diet			
Crude protein	*14%*	*14%*	*18%*	*18%*
Ingredient	*A*	*B*	*C*	*D*
Corn	81.35	81.35	70.70	70.70
Soybean meal	12.84	12.84	24.30	24.30
Soybean oil	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.10	1.10	1.00	1.00
Calcium carbonate	1.10	1.10	1.10	1.10
Vitamin premix[a]	0.10	0.10	0.10	0.10
Trace mineral premix[b]	0.34	0.34	0.34	0.34
L-Lysine HCl	0.55	0.55	0.15	0.15
DL-Methionine	0.07	0.07	0.01	0.01
L-Tryptophan	0.06	0.06	0.01	0.01
L-Threonine	0.19	0.19	0.00	0.00
Folic acid	0.01	0.01	0.01	0.01
Corn starch[c]	1.00	0.00	1.00	0.00
Cellulase[d]	0.00	0.07	0.00	0.07
Hemicell D[e]	0.00	0.10	0.00	0.10
Alpha-Gal 600 L[f]	0.00	0.30	0.00	0.30
Avizyme[g]	0.00	0.53	0.00	0.53
Chromic oxide	0.30	0.30	0.30	0.30

[a]Provided the following (per kg of diet): choline, 121 mg; niacin, 16.5 mg; pantothenic acid, 12.1 mg; riboflavin, 4.4 mg; vitamin B_{12}, .02 mg; vitamin E, 44.1 IU; vitamin A, 3310 IU; vitamin D, 331 IU; vitamin K, 2.21 mg. Supplied by Archer Daniels Midland Company, Effingham, IL.

[b]Provided the following (per kg of diet): sodium, 2.8 g; chloride, 2.8 g; copper, 7.79 mg; iodine, .34 mg; iron, 87.4 mg; manganese, 19.4 mg; selenium, 0.29 mg; zinc, 97.2 mg. Supplied by Archer Daniels Midland Company, Effingham, IL.

[c]Clinton® Brand, Archer Daniels Midland Company, Effingham, IL

[d]Sigma Chemical Company, provided 3,400 cellulase units per kg diet.

[e]ChemGen, provided 165,000 hemicellulase units per kg diet.

[f]Novo Nordisk, provided 1,800 alpha-galactosidase units per kg diet.

[g]FinnFeeds, provided 2,128 amylase units; 21,280 protease units; and 1,596 xylanase units; per kg diet.

Table 2. Apparent fecal digestibilities of dry matter, organic matter, crude protein and fecal output of the experimental treatments

	Treatment				
Item	*A*	*A plus steeping*	*B*	*B plus steeping*	*SEM*[a]
Dry matter, %	86.4[b]	86.3[b]	86.5[b]	86.1[b]	0.57
Organic matter, %	88.4[b]	88.4[b]	87.6[b]	87.0[b]	0.76
Crude protein, %	81.4[b]	79.5[b]	82.2[b]	81.4[b]	1.28
Fecal output (dry), g/d	218.9[b]	205.7[b]	210.7[b]	193.7[b]	13.65
Fecal output (wet), g/d	537.0[b]	586.0[b]	524.0[b]	516.0[b]	66.76
	Treatment				
Item	*C*	*C plus steeping*	*D*	*D plus steeping*	*SEM*[a]
Dry matter, %	87.0[b]	86.1[b]	86.0[b]	86.3[b]	0.47
Organic matter, %	89.1[b]	88.4[b]	88.6[b]	88.7[b]	0.63
Crude protein, %	84.5[b]	82.7[b]	84.2[b]	83.4[b]	0.98
Fecal output (dry), g/d	239.3[b]	248.2[b]	220.4[c]	248.0[b]	5.12
Fecal output (wet), g/d	639.5[b]	645.0[b]	519.0[b]	617.0[b]	29.00

[a]Standard error of the mean (n=4).
[b,c]Means in the same row with different superscript letters differ ($P < .05$).

Effect of the Halothane and Rendement Napole Genes on Carcass and Meat Quality Characteristics of Pigs.

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Introduction

Meat quality is affected by a number of factors such as environment, nutrition and genetics. The Halothane and RN genes impact animal performance, carcass composition and pork quality. The Halothane gene offers producers a rapid way of producing lean, heavily muscled market hogs that will receive higher packer premiums. However, Halothane reactor and carrier animals have poorer meat quality and processing characteristics, when compared to Halothane negative pigs. The meat quality problems associated with the Halothane gene result from a high incidence of the pale, soft, exudative (PSE) condition. Animals that carry the dominant allele of the Rendement Napole gene (RN^-) have been found to produce paler meat with reduced water holding capacity and processing yields. However, RN^- carriers have also been found to have higher carcass lean meat percentages and lower shear force values, indicating more tender meat. Thus, both the Halothane and RN genes independently have both negative and positive effects on carcass and meat quality and because of their different modes of action may have a greater combined effect on meat quality. However, there have been no studies that have characterized the impact of both genes in combination. Thus, the objective of this study was to determine the effects of and potential interactions between the Halothane and Rendement Napole genes on carcass and meat quality characteristics in pigs.

Materials and Methods

Animals for this study were bred and reared at the Moorman Swine Farm at the University of Illinois. Semen from Halothane carrier Hampshire sires (n = 9) was obtained from three boar studs and used to artificially inseminate dams (10 dams/sire) that were homozygous normal at both the Halothane and RN loci (NN/rn^+rn^+).

Biopsy sampling, Halothane genotyping and glycolytic potential determination. At approximately 40 kg live weight, a total of 200 pigs had a sample of longissimus removed using spring loaded biopsy equipment. The skin was trimmed from the sample and was used for the determination of Halothane genotype, using the method outlined by Fujii et al. (1991). Glycolytic potential on the longissimus sample was determined by enzymatic assay. Metabolite concentrations for glucose, glucose-6-phosphate, glycogen and lactate were determined. Glycolytic potential was calculated using the formula described by Monin and Sellier (1985): Glycolytic potential (umoles/g wet tissue) = 2([glycogen] + [glucose-6-phosphate] + [glucose]) + [lactate].

A frequency distribution for the glycolytic potential values for the population was plotted and animals were classified as having low or high glycolytic potential on the basis of this distribution.

Animals: Boars that sired litters with both a bimodal distribution for glycolytic potential and with both Halothane carrier and normal pigs were considered to be heterozygous for the RN and

Halothane genotypes ($Nn/RN^{-}rn^{+}$). Four of the original nine boars were found to be heterozygous at both loci and only progeny from these sires and litters with at least eight pigs were used in this study. Animals were classified into four genotypes on the basis of the results of the Halothane test and the glycolytic potential values: 1) Halothane and RN normal ($NN/rn^{+}rn^{+}$) (n = 31); 2) Halothane carrier and RN normal ($Nn/rn^{+}rn^{+}$) (n = 27); 3) Halothane normal and RN carrier ($NN/RN^{-}rn^{+}$) (n = 30); and 4) Halothane carrier and RN carrier ($Nn/RN^{-}rn^{+}$) (n = 23).

Trial design. Four genotypes and two sexes were evaluated in a 4 × 2 factorial arrangement with a total of 111 animals being used. At 120 kg live weight, animals were taken off feed and transported to a commercial slaughter facility which was located approximately 150 miles from the farm. Animals were weighed before loading and mixed in one group during transport and in the lairage. At the slaughter plant, pigs were held for approximately 16 h prior to slaughter without food but with access to water. Slaughter and carcass dressing was carried out using standard commercial procedures.

Carcass and meat quality evaluation. At 24-h postmortem, cold carcass weight was recorded, and standard carcass measurements were obtained from the left side of each carcass. Longissimus pH was measured at 45 min and 24-h postmortem. Subjective scores for longissimus color, firmness, and marbling were taken on the cut surface of the loin at the tenth rib using the procedures described by NPPC (1991) using five point scales (1 = pale, soft, and devoid of marbling; 5 = dark, firm, and moderately abundant or greater marbling). Minolta color (L*, a*, and b* values) was measured on the cut surface of the loin section at the 10^{th} rib. Two loin chops (2.5 cm thick) were cut from the longissimus immediately posterior to the 10^{th} rib with one chop being used to determine drip loss and the other for chemical analysis. A section of the loin was taken immediately anterior to the last rib for shear force and cooking loss evaluation. The section was vacuum packaged, aged for 7 d at 4°C, cut into 2.5 cm thick chops, and frozen (-20°C).

Shear force and cooking loss. A chop was removed from the frozen loin section for Warner-Bratzler shear force determination, thawed for 24 h at 4°C, and cooked to an internal temperature of 70°C. The chops were weighed before and after cooking to determine cooking loss. Shear force was measured on three cores from each chop.

Results

Cluster analysis indicated that the breakpoint between the two parts of the bimodal distribution for loin glycolytic potential was at 225 umol/g. Animals (n = 55) with glycolytic potential greater than 225 umol/g were considered to be carriers of the RN^{-} allele ($RN^{-}rn^{+}$), while pigs (n = 56) with glycolytic potential less than 225 umol/g were considered to be homozygous normal ($rn^{+}rn^{+}$).

RN gene effects. No differences were found between RN carrier (_ _/$RN^{-}rn^{+}$) and normal (_ _/ rn^{+} rn^{+}) animals for carcass measurements (Table 1). Few studies have reported the effects of the RN gene on carcass characteristics and the results of these are conflicting, with some showing a reduced backfat thickness and increased carcass lean content for pigs with the dominant allele (LeRoy et al., 1996) and others showing no effect of the RN gene (Enfalt et al., 1997a; Enfalt et al., 1997b). In the

current study, pigs carrying the RN^- allele (_ _/RN^-rn^+) had significantly lower ultimate pH, higher longissimus L* and b* values, and greater drip loss and cooking loss than homozygous recessive animals (_ _/rn^+rn^+; Table 2).

Halothane gene effects. No difference ($P > .05$) was observed for dressing percentage between Halothane carrier and normal genotypes (Table 1). Halothane carrier (Nn/_ _) animals had significantly shorter carcasses than Halothane normal (NN/_ _) animals. Backfat thickness measurements did not differ among the Halothane genotypes. Halothane carrier pigs had lower longissimus ultimate pH compared to normal animals (NN/_ _) (Table 2) which is consistent with other studies in the literature (Jones et al., 1994; Leach et al., 1996). In contrast, Pommier et al. (1998) and Tam et al. (1998) found no significant differences in ultimate pH between Halothane genotypes. Halothane carriers (Nn/_ _), in comparison to homozygous normal animals (NN/_ _), had higher L*, b* and drip loss values (Table 2), indicating paler meat with less water-holding capacity.

RN and Halothane interaction effects. There were no significant RN × Halothane interactions for any of the carcass measurements. However, the interaction between RN and Halothane genotype was significant for subjective color, firmness and marbling scores (Table 2), suggesting that the effects of the two genes were not additive for these traits. Pigs that were normal for both genes (NN/rn^+rn^+) had higher values for subjective color, firmness and marbling (Table 2), indicating darker, firmer muscle compared to the other three genotypes, which were similar in these respects. Thus, the detrimental alleles of the two genes, either singly or in combination, resulted in similar subjective longissimus color, firmness and marbling. In contrast, the Halothane by RN gene interaction was not significant for ultimate pH, Minolta L* values, or drip loss (Table 2) suggesting that effects of the genes were additive for these traits and that animals that were carriers of both detrimental alleles produced the meat with the lowest ultimate pH, the lightest color, and the greatest drip loss of all of the genotypes evaluated. There was a RN × Halothane interaction ($P < .05$) for shear force (Table 2) with pigs that were homozygous normal at both loci (NN/rn^+rn^+) having the highest values suggesting tougher meat compared to the three genotypes, which were similar in this respect.

Sex effects. There were no differences between the sexes for carcass weight, dressing percentages or carcass length (Table 1). Gilts had less backfat at the 10^{th} rib, and last lumbar vertebra and greater loin eye depth and area compared to barrows. These results are similar to most other studies (Ellis et al., 1996; Leach et al., 1996). Gilts had lower subjective marbling scores than barrows (Table 2). Minolta b* values were higher for barrows (Table 2); however, there were no differences between the sexes for any other meat quality traits. Other studies have generally found little difference in muscle quality between barrows and gilts (Barton-Gade, 1987; Leach et al., 1996).

Conclusions

1. Gilts have significantly less backfat and greater loin eye area when compared to barrows with no difference in meat quality measurements.

2. This study suggest that the RN and Halothane genes have little or no effect on carcass leanness or dressing percent. However, other studies have reported slight advantages in these carcass measurements for RN and Halothane animals.

3. The RN and Halothane genes independently have negative effects on muscle color and water-holding capacity. The lack of any interaction between the two genes for longissimus ultimate pH, objective color, and drip loss suggest that these genes in combination may have a greater effect on pork quality.

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Table 1. Least square means for RN and Halothane genotypes and sex for slaughter and carcass measurements

Variable	RN genotype		Halothane genotype		Sex		AVG SE	Level of Significance[a]			
	_ _/rn^+rn^+	_ _/RN^- rn^+	NN/_ _	Nn/_ _	Barrows	Gilts		RN	Hal	Sex	RN × Hal
Slaughter weight, kg	119.2	118.2	118.8	118.6	121.7	115.7	3.10	NS	NS	***	NS
Cold carcass weight, kg	87.2	86.9	87.4	86.7	87.5	86.6	1.14	NS	NS	NS	NS
Dressing percentage	73.8	73.6	74.1	73.4	74.1	73.3	1.12	NS	NS	NS	NS
Carcass length, cm	82.3	82.2	82.7	81.8	82.3	82.2	.44	NS	*	NS	NS
Backfat depth, mm											
Tenth rib	23.5	22.6	23.0	23.1	26.0	20.2	1.16	NS	NS	***	NS
Loin eye area, cm^2	43.7	43.3	42.6	44.5	41.5	45.5	1.36	NS	NS	***	NS

[a] NS, *, **, *** = not significant, $P < .05$, $P < .01$, $P < .001$, respectively.

Table 2. Least square means for RN and Halothane genotypes and sex for meat quality measurements taken on the longissimus

	RN genotype		Halothane genotype		Sex		AVG	Level of Significance[c]			
Variable	_ _/rn$^+$rn$^+$	_ _/RN$^-$rn$^+$	NN/_ _	Nn/_ _	Barrows	Gilts	SE	RN	Hal	Sex	RN × Hal
Ultimate pH	5.50	5.26	5.45	5.32	5.37	5.39	.044	***	***	NS	NS
Color[d]					2.12	2.01	.149	*	*	NS	**
NN/_ _	2.60[b]	1.85[a]					.181				
Nn/_ _	1.88[a]	1.95[a]					.192				
Firmness [d]					2.18	2.09	.149	NS	*	NS	*
NN/_ _	2.53[b]	2.10[a]					.182				
Nn/_ _	2.03[a]	1.89[a]					.182				
Marbling[d]					1.80	1.52	.124	NS	*	*	**
NN/_ _	2.11[b]	1.53[a]					.153				
Nn/_ _	1.44[a]	1.55[a]					.152				
Minolta L*	50.29	54.50	50.69	54.10	53.42	51.38	1.350	***	***	NS	NS
Minolta a*	8.75	8.71	8.76	8.71	9.00	8.47	1.230	NS	NS	NS	NS
Minolta b*	7.28	9.13	7.51	8.90	8.72	7.69	1.061	***	***	NS	NS
Drip loss, %	4.67	7.02	5.11	6.59	6.08	5.62	.892	***	**	NS	NS
Cooking Loss, %	23.99	26.26	24.61	25.65	24.46	25.80	.770	**	NS	NS	NS
Warner Bratzler Shear, kg					3.83	4.09	.121	*	NS	NS	*
NN/_ _	2.11[b]	1.53[a]					.172				
Nn/_ _	1.44[a]	1.55[a]					.171				

[ab] Means with differing superscripts for a RN and Halothane interaction differ ($P < .05$).
[c] NS, *, **, *** = not significant, $P < .05$, $P < .01$, $P < .001$, respectively.
[d] Subjective color, firmness, and marbling scores, where 1 =pale, soft and devoid of marbling and 5=dark, firm and moderately abundant marbling.

The Effect of the Time of Feeding Prior to Slaughter of Supplemental Magnesium Sulfate Heptahydrate on Pork Quality.

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Introduction

Australian research has shown that including magnesium aspartate in the diet of pigs during the final five days of the finishing period before slaughter reduced the incidence of Pale, Soft, and Exudative (PSE) pork thus improving the color and water holding capacity of the pork (D'Souza et al., 1998). In a follow up study (D'Souza et al., 1999), magnesium sulfate and magnesium chloride were found to be as effective as magnesium aspartate in reducing both the incidence of PSE and muscle drip loss. However, these studies were carried out with entire males slaughtered at relatively light weights (less than 90 kilogram live weight). Research is required to validate the effect of magnesium supplementation on pork quality under US production conditions which includes castrated males and heavier slaughter conditions.

Thus, the objective of this study was to compare the effect of time feeding before slaughter of a fixed level of supplemental magnesium on pork quality.

Materials and Methods

Study design. The study was carried out as a completely randomized design with four dietary treatments:

Control:	Control diet-no added magnesium sulfate heptahydrate
5 d:	Magnesium sulfate heptahydrate-fortified diet fed for 5 days pre-slaughter
3 d:	Magnesium sulfate heptahydrate-fortified diet fed for 3 days pre-slaughter
2 d:	Magnesium sulfate heptahydrate-fortified diet fed for 2 days pre-slaughter

The magnesium sulfate-fortified diet was formulated to supply 3.2 grams of magnesium per pig per day (assuming a daily feed intake of 2.72 kilograms/pig and that the magnesium content of sulfate was 9.6% as analyzed). Diet composition and calculated analysis are presented in Table 1.

Animals. A total of 144 pigs were used in the study which was carried out in three replicates with 36 pigs in each of replicates 1 and 2 and 72 pigs in replicate 3. The pigs used were the progeny of PIC line 326 sires mated to C22 dams (Replicates l and 2) and PIC line 406 sires mated to C22 dams (Replicate 3). Barrows (n = 50) and gilts (n = 94) were balanced across magnesium treatments. Prior to the start of the study, pigs were reared under standard commercial conditions. Pigs for each replication were selected one week prior to the start of the study period at approximately 105 kilograms live weight. They were allotted to treatment on the

basis of sex, weight, Rendement Napole (RN) genotype, Halothane genotype and feed intake (determined in the acclimation phase).

RN and Halothane genotype determination. The pigs used in replicates 1 and 2 were from a population within which the RN gene was segregating. Animals used in replicate 3 were from a population within which the RN and Halothane genes were segregating. Both of these genes are known to have a major impact on pork quality and, consequently, only Halothane negative pigs were selected for replicate 3. Pigs were allotted across treatments on the basis of RN genotype for all replicates. The RN and Halothane genotypes were determined from a biopsy sample of skin, fat and muscle taken from the longissimus at approximately 40 kg live weight using spring loaded biopsy equipment (Biotech PPB-U, Nitra, Slovakia). The muscle sample was used to determine glycolytic potential which is the basis for predicting RN genotype while the skin was used to determine Halothane genotype. A frequency distribution for the glycolytic potential values for the population tested was plotted and animals were classified as having low or high glycolytic potential on the basis of this distribution. Samples with glycolytic potential greater than 200 μmol/g for replicates 1 and 2 and greater than 210 μmol/g for replicate 3 were considered to be RN carriers (RN^-rn^+); those with glycolytic potentials below 200 μmol/g for replicates 1 and 2 and below 210 μmol/g for replicate 3 were considered to be homozygous normal (rn^+rn^+) for the RN gene.

Housing and Feeding. Animals were housed in one of two identical buildings with 36 individual pens in each building. The environment within the building was controlled using a thermostat and fan ventilation. Pen dimensions were .91 x 1.83 meters giving a floor space allowance of 1.67 meters^2/pig. Pigs were moved into the building seven days prior to the start of the study. Feed in the form of a standard pelleted finisher diet (Control diet; Table 1) was offered ad libitum prior to the start of the study from a single-space feeder and water was freely available from a nipple waterer. A restricted feeding regime was used with pigs being given 2.75 kg/pig/day throughout the trial period.

The same finisher diet formulation used in the acclimation period was used for all treatments during the trial period (Table 1). Magnesium sulfate heptahydrate was used as the magnesium source. The magnesium-supplemented diet was substituted for the control diet on days 1, 3 and 4 of the trial period for the five-, three-, and two-day treatments, respectively. Pigs on the control treatment were fed the control diet for the entire trial period; pigs on the five-day treatment were fed the magnesium diet on days 1 to 5 of the trial period; pigs on the three-day treatment were fed the control diet on days 1 and 2 and the magnesium diet on days 3 to 5 of the trial period; pigs on the two-day treatment were fed the control diet for days 1 to 3 and the magnesium diet on days 4 and 5 of the trial period. The pigs on the magnesium sulfate heptahydrate-fortified diets were offered the experimental diet at 6:00 am on the day they started their respective treatment. During the experimental period, feed additions and refusals were measured on a daily basis. All pigs on test were weighed at the start of each feeding period (days 1, 3, and 4) and at end of the experimental period immediately prior to transport for slaughter. All pigs were health checked and fecal consistency scores were taken (1 = normal; 2 = slight

looseness; 3 = moderate looseness; 4 = significant scouring) on a daily basis. General comments concerning pig behavior were also recorded throughout the trial period.

Slaughter and carcass evaluation. Slaughter was performed at a commercial packing facility with animals being transported to the plant on the morning of the final day of the experimental period. Pre-slaughter handling was standardized and reflected commercial conditions. Pigs were mixed and loaded onto a livestock trailer at 6:00 am, and transported directly to the packing facility. Slaughter took place on the same day as soon as possible after arrival at the plant. Pigs for all replicates were taken to the kill floor approximately 15 minutes after arrival in order to minimize the time in lairage. Hot carcass weights and carcass Fat-o-Meater measurements were collected on the slaughter line.

Following overnight chill, carcass measurements were taken, including carcass length (measured from the cranial tip of the aitch bone to the cranial edge of the first rib adjacent to the thoracic vertebra), midline fat measurements (opposite the first rib, last rib, and last lumbar vertebra), 10^{th} rib fat depth (measured at one-half the distance from the midline) and loin eye area at the 10^{th} rib.

At 24-h postmortem the loin from the left side of each carcass was removed and transported to the University of Illinois Meat Science Laboratory for meat quality assessment. Subjective color, firmness, and marbling were evaluated on the cut surface of the longissimus at the 10^{th} rib using the procedures of NPPC (1991), based on five point scales (1 = pale, soft and devoid of marbling; 5 = dark, firm, and moderately abundant or greater marbling). Hunter color (L*, a*, and b*) was also measured on the cut surface of the loin section at the 10^{th} rib using a Hunter LabScan Spectrocolormeter. Two loin chops were cut from the longissimus immediately posterior to the 10^{th} rib and trimmed of epimysium and external fat. One chop was weighed, placed in a Whirl-pak bag, suspended in a cooler (4°C) for 48 hours, reweighed, and drip loss was recorded.

A 5-gram sample was removed from the second chop, homogenized in 15 milliters of distilled water, and the pH measured. The remainder of the chop was homogenized, placed in a Whirl-pak bag, and frozen (-20°C) for subsequent proximate analysis. The remaining loin sections (anterior and posterior to the 10^{th} rib) were weighed, vacuum packaged, and stored in a cooler (4°C) for six days, reweighed and purge loss calculated.

Results and Discussion

The results relating to the diet composition, growth performance, carcass characteristics, and meat quality for all animals used in the study are presented in Table 1, 2, 3 and 4, respectively. Generally speaking, the treatment effects were similar across the replicates and, therefore, only the combined results for all replicates will be discussed in this report.

Magnesium Treatment Effects. Diets were analyzed in duplicate for crude protein, lysine, calcium, phosphorus, and magnesium. Both diets were similar in crude protein, lysine, calcium and phosphorus. Analyzed magnesium levels in the control and magnesium sulfate-

supplemented diets were .12% and .24%, respectively (Table 1). This equates to a daily magnesium intake of 3.3 and 6.6 g for pigs on the control and magnesium-sulphate supplemented diets, respectively.

No differences were found between magnesium treatments for growth performance (Table 2) or carcass characteristics (Table 3). Barrows had heavier hot carcass weights, and were fatter at the 10^{th} rib and last lumbar vertebrae than gilts, results that are in line with most other studies. Treatment and sex means for fresh meat quality measurements are presented in Table 4. No interactions between magnesium treatment and sex were detected for any of the meat quality measurements. There were no treatment differences for ultimate pH, marbling scores, Hunter a* and b* values, and purge loss. Pigs on the three-day supplementation treatment had lower subjective color scores, indicating paler meat, compared to the other treatments which were similar in this respect. Subjective firmness scores were lower for pigs on the control and three-day treatments indicating softer muscle compared to the other two treatments.
Relative to controls, feeding the magnesium-supplemented diets for five and two days prior to slaughter resulted in a decrease in Hunter L* values. In addition, drip loss was reduced for all of the periods of magnesium supplementation compared to the control treatment (Table 4).

Rendement Napole Gene Effects. Generally speaking, the effects of the RN gene found in this study were similar to those observed in most other research carried out at this and other centers. The was no effect of the gene on growth performance (Table 2). Backfat thickness at the last lumbar vertebra and tenth rib was lower for RN carriers (RN^-rn^+) than for homzygous recessive animals (rn^+rn^+; Table 3). In addition, RN carriers had lower ultimate pH and increased purge and drip loss compared to homozygous recessive pigs (Table 4). Most other studies have shown that the dominant allele of the RN gene (RN^-) is associated with a lower muscle pH and reduced water holding capacity.

There were no statistically significant interactions between magnesium treatment and RN genotype for any of the variables measured in this study (Tables 2, 3 and 4) suggesting that the improvements in pork quality resulting from supplementary magnesium would be similar across the range of RN genotypes currently found in the US industry.

General observations. As a consequence of the restricted feeding regime that was used in this study, all pigs consumed the majority of their daily feed allowance in the morning, resulting in an average time off feed prior to slaughter of approximately 18 to 24 hours. No differences between the treatments were observed in the general health of the animals and the fecal consistency was considered normal for all animals throughout the study period.

Conclusions.
1. The results of this study suggest that the feeding of a fixed level of magnesium (3.3 g/pig/day) to finishing pigs for periods of up to five days prior to slaughter has no effect on animal health, fecal consistency, growth performance or carcass characteristics.

2. Feeding supplemental magnesium sulfate heptahydrate for periods of two, three, and five days resulted in a reduction in drip loss; objective muscle reflectance (Hunter L*) was reduced for the five- and two-day treatments only.

3. Despite these improvements in color and water holding capacity, purge loss, as measured in this study, was not influenced by magnesium supplementation, a result that requires further investigation.

4. The lack of a statistical interaction between magnesium treatment and RN genotype suggests that the improvement in pork quality resulting from the supplemental magnesium treatment would be similar for all RN genotypes. The RN gene has only been observed in the Hampshire breed where the incidence of the dominant allele is generally high in US populations. This study suggests that any beneficial effect of magnesium supplementation will be independent of the RN gene status of the population in which it is used.

5. Overall, these results support the hypothesis that feeding supplemental magnesium (at a level of 3.3 g/pig/day) for periods between two and five days prior to slaughter can improve pork color and drip loss. However, further studies are required to establish the optimum combination of level of supplementation and time of feeding to improve these quality attributes.

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Table 1. Percentage composition[a] and calculated analysis of experimental finisher diets.

	Control Diet		Magnesium Sulfate Diet	
Ingredient				
Corn	81.12		79.65	
Soybean meal (dehulled)	15.70		15.95	
Fat	1.00		1.00	
Limestone	0.85		0.85	
Di-calcium phosphate	0.80		0.80	
Trace mineral salt[b]	0.30		0.30	
Vitamin mix[c]	0.10		0.10	
L-Lysine HCl	0.13		0.13	
Magnesium sulfate heptahydrate	-		1.22	
Composition	Calculated	Analyzed	Calculated	Analyzed
Crude protein, %	14.55	13.75	14.55	14.38
Lysine, %	.787	.83	.791	.89
Calcium, %	.55	.56	.55	.55
Phosphorous, %	.21[d]	.52[e]	.21[d]	.52[e]
Magnesium, %	-	.12[f]	-	.24[f]
ME, kcal/kg	3,346	-	3,305	-

[a] As-fed basis.

[b] Each kilogram of mix contained the following: Se, 85.7 mg; I, 100 mg; Cu, 2.3 g; Mn, 5.7 g; Fe, 25.7 g; Zn, 28.6 g; NaCl, 855 g.

[c] Each kilogram of mix contained the following: vitamin A, 3,000,000 IU; vitamin D3, 330,000 IU; vitamin E, 44,000 IU; vitamin K, 2.2 g; vitamin B12, 17.9 mg; riboflavin, 4.4 mg; d-pantothenic acid, 12.1 g; niacin, 16.5 g; choline chloride, 165 g; and roughage products to 1 kg.

[d] Expressed as % of available phosphorus.

[e] Expressed as % of total phoshorus.

[f] % magnesium determined using an Inductively Coupled Plasma (ICP) spectrometry assay.

Table 2. Least square means of growth performance for replicates 1,2 and 3 (barrows and gilts n = 139).

	Dietary Treatment[a]						Sex				RN Genotype			
VARIABLE	Control	5 day	3 day	2 day	Ave SE	P Value	Barrows	Gilts	Ave SE	P Value	rn^+rn^-	RN^+rn^-	Ave SE	P Value
Start weight[b], kg	122.7	121.3	122.1	119.4	1.53	.42	122.0	120.7	1.13	.44	123.0[f]	119.7[e]	1.12	.03
End weight[c], kg	124.9	123.8	125.2	122.2	1.43	.41	124.2	123.9	1.05	.86	125.3	122.7	1.04	.07
Average daily gain[d], kg	.465	.504	.629	.578	.0945	.61	.433[e]	.655[f]	.0696	.03	.474	.614	.0690	.14
Average daily feed intake, kg	2.72	2.75	2.75	2.75	.010	.07	2.74	2.75	.007	.40	2.75	2.74	.007	.13

[a] Dietary treatment: Control = standard finisher diet fed 5 days before slaughter.
5 Day = magnesium diet fed 5 days before slaughter.
3 Day = magnesium diet fed 3 days before slaughter.
2 Day = magnesium diet fed 2 days before slaughter.

[b] Start of trial period (Day 1).

[c] End of trial period (Day 6 before shipping).

[d] Calculated using start and end weights from trial period.

[ef] Means with common superscript do not differ (P>.05).

Table 3. Least square means of carcass traits for replicates 1,2 and 3 (barrows and gilts, n = 139).

	Dietary Treatment[a]						Sex				RN Genotype			
VARIABLE	Control	5 day	3 day	2 day	Ave SE	P Value	Barrows	Gilts	Ave SE	P Value	rn^+rn^+	RN^-rn^+	Ave SE	P Value
Slaughter weight, kg	126.4	124.3	125.1	124.0	1.37	.60	126.8[d]	123.1[c]	1.01	.02	126.7[d]	123.2[c]	1.00	.01
Hot carcass weight, kg	93.8	94.0	94.2	92.5	1.29	.74	96.1[d]	91.2[c]	.97	.00	94.9	92.4	.96	.06
Dressing percent	74.2	74.9	74.7	74.5	.58	.80	75.0	74.2	.44	.25	74.8	74.4	.43	.43
Carcass length, cm	85.5	84.2	83.8	84.5	.54	.16	84.3	84.7	.40	.45	84.5	84.4	.40	.83
First rib backfat, cm	4.14	3.87	4.12	4.09	.185	.71	4.16	3.96	.139	.35	4.16	3.96	.139	.40
Last rib backfat, cm	2.83	2.90	2.87	2.69	.136	.65	2.92	2.72	.102	.20	2.89	2.76	.100	.35
Last lumbar backfat, cm	2.01	2.16	1.99	2.27	.123	.26	2.23	1.98	.091	.06	2.23[d]	1.98[c]	.090	.04
10^{th} rib backfat, cm	2.22	2.48	2.17	2.21	.127	.30	2.45[d]	2.09[c]	.095	.01	2.43[d]	2.11[c]	.094	.01
Loin eye area, cm^2	40.76	40.96	42.99	41.04	.886	.22	40.70	42.17	.654	.13	41.09	41.78	.648	.44
Carcass muscle score[b]	2.1	2.1	2.3	2.2	.09	.26	2.2	2.2	.06	.88	2.2	2.2	.06	.78

[a] Dietary treatment: Control = standard finisher diet fed 5 days before slaughter
5 Day = magnesium diet fed 5 days before slaughter
3 Day = magnesium diet fed 3 days before slaughter
2 Day = magnesium diet fed 2 days before slaughter

[b] Carcass muscle score: 1=thin to 3=thick

[cd] Means with common superscript within the same row do not differ (P>.05).

Table 4. Least square means of fresh meat quality traits in the longissimus for replicates 1,2 and 3 (barrows and gilts, n = 139).

	Dietary Treatment[a]						Sex				RN Genotype			
VARIABLE	Control	5 day	3 day	2 day	Ave SE	P Value	Barrows	Gilts	Ave SE	P Value	rn^+rn^+	RN^-rn^+	Ave SE	P Value
Ultimate pH	5.38	5.43	5.40	5.42	.023	.33	5.41	5.40	.017	.76	5.45^d	5.36^c	.017	.00
Color[b]	$2.65^{c\,d}$	2.81^d	2.39^c	2.76^d	.112	.02	2.66	2.64	.082	.90	2.70	2.60	.08	.39
Firmness[b]	2.02^c	2.42^d	$2.20^{c\,d}$	2.58^d	.124	.01	2.23	2.38	.091	.25	2.37	2.25	.091	.33
Marbling[b]	2.18	2.19	2.09	1.99	.155	.76	2.22	1.99	.114	.18	2.11	2.11	.113	.97
Hunter L*	55.7^d	$53.9^{c\,d}$	55.5^d	52.8^c	.738	.02	54.8	54.2	.54	.46	54.1	54.9	.54	.26
Hunter a*	7.4	7.3	7.3	7.7	.22	.51	7.4	7.4	.16	.85	7.4	7.4	.16	.70
Hunter b*	15.3	14.9	15.1	15.0	.22	.69	15.2	15.0	.16	.33	15.1	15.1	.16	.91
Drip loss, %	8.98^d	7.41^c	7.89^{cd}	7.29^c	.447	.04	7.86	7.93	.329	.89	7.43^c	8.35^d	.327	.04
Purge loss, %	2.12	2.01	2.25	2.03	.131	.51	2.04	2.16	.096	.40	1.91^c	2.28^d	.096	.01

[a] Dietary treatment: Control = standard finisher diet fed 5 days before slaughter
5 Day = magnesium diet fed 5 days before slaughter
3 Day = magnesium diet fed 3 days before slaughter
2 Day = magnesium diet fed 2 days before slaughter

[b] Subjective score: 1 = extremely pale, soft, and devoid of marbling to 5 = extremely dark, firm, and abundant marbling

[c d] Means with common superscript within the same row do not differ (P>.05)

Effects of group size, floor-space allowance, and feeder placement on pig performance in the nursery[1]

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Introduction

Modern pig housing systems are expensive to construct and operate. Historically, the number of pigs per pen (i.e. group size) and the floor area allowed per pig (i.e. stocking density) have been key factors in the design and use of pig houses. Historically, pigs typically have been penned from weaning to market weight in groups of 10 to 30 animals. Now, however, group sizes of 50 to 100 or even more are being advocated by some as a management strategy which is claimed to minimize housing cost, maximize housing use, and improve overall profitability.

Research has demonstrated that pig performance in the nursery stage varies with number of animals per group and floor-space allowance (NCR-89, 1984; McConnell et al., 1987; Spicer and Aherne, 1987; Kornegay et al., 1993). Housing pigs at reduced floor space and/or at increased group size has been shown to have a negative influence on feed intake and growth rate (Kornegay and Notter, 1984). Curtis (1996) suggested that the effect of group size on voluntary feed intake may be due to greater "social tension" with increases in group size resulting from competition for access to feeders. Therefore, allowing pigs kept in large groups access to multiple feeding locations compared to those with just one location may improve feed intake and weight gain, as a result of reducing the effect of social rank during feeding activity (Hansen et al., 1982).

McGlone and Newby (1994) observed no differences in growth rate in grow-finish pigs in groups of 40, 20, or 10 when pigs were kept at constant floor-space allowance (.74 m^2/pig). They analyzed pig postures over a 24-h period via time-lapse video records to determine "free space" (i.e. the total floor space not occupied by any pig at any particular moment). "Free space" increased with group size under constant floor-space allowance. In a second experiment, none, half, or all "free space", respectively, was removed from pens holding groups of 20 pigs. Removing all "free space" had a detrimental effect on pig performance, but removing half did not. Therefore, where large group sizes are employed in commercial operations, perhaps total space per pig can be decreased without reducing growth rate. However, for relatively large group sizes (i.e. 100 pigs) there is a paucity of data related to effects of and interactions between group size and floor-space allowance on pig performance.

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Two experiments were conducted to evaluate the effect of pen design on nursery pig performance. Experiment 1 was conducted to quantify effects of and interactions between group size (20 versus 100) and space allowance on weanling pig performance. Experiment 2 was conducted to evaluate the effects of feeder location (multiple versus single) in large groups on the performance of weanling pigs.

Experiment 1

Materials and Methods. This experiment was conducted to assess effects of two group sizes (20 [small] or 100 [large] pigs per pen) and two floor-space allowances (calculated requirement [adequate] and calculated requirement less 50% of calculated "free space" [reduced]) on performance of weanling pigs. The experiment was carried out in two trials, each trial having four replicates. The research was carried out at the Burton Russell Swine Research Farm, United Feeds, Inc., Frankfort, IN.

Floor-space allowance was kept constant for the first 4 wk post-weaning and for the period from wk 5 through 9 and was based on body weight using the formula (Petherick and Baxter, 1981): floor-space allowance (m^2) = $k \times BW^{.667}$, where BW is body weight in kg and k is equal to a constant. The value assumed for k was 0.030 (Edwards and Armsby, 1988; Gonyou and Stricklin, 1998). The body weight assumed in these calculations were 14 kg and 45 kg which were the predicted end weights for wk1 through wk 4 and wk 5 through 9, respectively. "Free space," as described by McGlone and Newby (1994), was calculated for both group sizes using the formula $Y = 0.179 + 0.002092X$, where Y is "free space" in m^2 and X is number of pigs/group. McGlone and Newby (1994) developed this formula using pigs of 93 kg live-weight; therefore, a ratio incorporating the estimated floor-space allowances for the body weight of the pigs assumed in our study (viz., 14 kg and 45 kg) were used to determine the "free-space" values used. The reduced floor-space allowance was estimated by subtracting half of the determined "free space" from the calculated requirement. Pen sizes were adjusted at the starts of wk 1 and wk 5 (Table 1).

Crossbred pigs (Bunge Line 5 × Bunge Line 13) were weaned at 16 ± .2 d of age and allotted to treatment at 22 h post-weaning. Pigs were randomly allotted to treatment pens on the basis of gender and weight.

Pigs were housed in an insulated, mechanically ventilated, curtain-sided nursery house with tri-bar flooring and were given ad libitum access to a six-phase dietary regimen formulated to meet or exceed NRC (1998) nutrient requirements (Table 2). Pens were equipped with one nipple drinker/10 pigs and a four-space nursery feeder/20 pigs, giving 4 cm of feeder-trough space/pig. Feeders were positioned in the center of the pen for all pen designs and were accessible from both sides (Figure 1). Water nipples were placed at equal intervals on one wall in each pen. Each room accommodated one replicate of the trial.

Air temperature was maintained at 22°C to 25°C using a thermostatically controlled heater and fan ventilation. During wk 1 post-weaning, supplemental heat was provided via propane brooders. Room temperature and relative humidity were measured and recorded.

Pig performance data were analyzed as a randomized block design using the GLM procedure of SAS (1990). Pen was considered the experimental unit. The model included effects of group size, space allowance, the interaction of group size and space allowance, trial, and replicate nested within trial.

Results and Discussion. Pig weight and variation in weight (as indicated by the coefficient of variation for each weigh period) are given in Table 3. Trial had an effect on pig weight, but there was no interaction between trial and other treatments. Feed intake data for wk 1 through 4 and growth-performance data for wk 1 through 9 are given in Table 4. There were no interactions between group size and floor-space allowance for any of the variables evaluated for either wk 1 through 4 or wk 1 through 9.

Effect of Group Size. Pigs in large groups were lighter at the end of wk 1, 4, and 9 post-weaning compared to pigs in small groups (Table 3). Variation in live weight was greater for the pigs kept in large groups at the end of wk 9 (Table 3). Pigs in large groups had lower ADG and ADFI during wk 1, 2 through 4, and wk 1 through 4 (Table 4). Although morbidity was less than 1% in all treatment subgroups, caretakers reported having increased difficulty in identifying and treating sick pigs in the large pens compared to small pens.

Similarly, Kornegay and Notter (1984) found that for weanling pigs (body weight < 30 kg) at a constant floor-space allowance, ADG and ADFI decreased as number of pigs per group increased (from 3 through 32 pigs). However, other studies have employed fewer pigs per pen than the current study to evaluate any effect of group size on pig performance during the grow-finish period, and results have been inconsistent. In growing pigs (20 to 60 kg), Petherick et al. (1989) observed a lower ADG for pigs in large groups (36 pigs/pen) compared to smaller (8 or 16 pigs/pen). Similarly, Gelhbach et al. (1966) reported decreased performance with increased number of pigs per pen (8 vs. 16 pigs) in the grower period. Randolph et al. (1981), however, found no performance differences between group sizes of 5 and 20 during the grow-finish period (20 to 90 kg). Hyun (1997) observed a lower ADG for pigs in groups of 12 compared to 2, 4, or 8 pigs per group during the growing period; however, similar ADG was observed for pigs penned in these group sizes during the finishing period. McGlone and Newby (1994) found similar performance by pigs in groups of 10, 20, and 40; however, pig injury and morbidity were increased for pigs in groups of 40.

Effect of Floor Space. At the end of wk 1 post-weaning, pigs given reduced floor-space allowance had similar body weight as those given adequate space (Table 3). However, pigs given the reduced floor space were .41 and 1.64 kg lighter than pigs given adequate floor space at the end of wk 4 and 9, respectively. Floor-space allowance did not affect variation in piglet weight at any point during the study (Table 3). This is in agreement with Kornegay et al. (1985)

who found no effect of floor-space allowance on variation in pig live weight. In addition, floor-space allowance did not affect ADG during wk 1 (Table 4). However, pigs given reduced floor space had lower ADG for wk 2 through 4, wk 4 through 9, and wk 1 through 9 (Table 4).

Kornegay and Notter (1984) observed that both ADG and ADFI increased with increasing floor-space allowance per pig, and a similar but smaller effect was found for feed conversion efficiency. In the current experiment, floor-space allowance did not affect ADFI during wk 1 through 4 (Table 4); however, G/F was higher during the period wk 1 through 4 for pigs with a greater floor-space allowance.

The effect of reducing 50% of the "free space" on performance was similar in large and small groups. This supports the hypothesis of McGlone and Newby (1994) that, as group size increases, it may be possible to reduce the total amount of floor space per pig without hindering performance. However, the relationship between group size and floor space was not directly tested in the current study, and further research is needed in this area.

Decreasing floor-space allowance per pig may lead to increased social tension and a higher incidence of aggressiveness in pigs (Ewbank and Bryant, 1972; Randolph et al.,1981; Curtis, 1996). In this experiment, however, there were no signs of tail- or ear-biting, as a result of either increasing group size or reducing floor-space allowance.

Experiment 2

Materials and Methods. This experiment was conducted to assess the effects of group size and feeder location on pig performance. The designs were: 1) large group size (100 pigs/pen) with multiple (five) feeder locations; 2) large group size (100 pigs/pen) with a single feeder location; and 3) small group size (20 pig/pen) with a single feeder. The experiment was carried out in two trials, each trial having four replicates. The research was conducted in the same facility as Experiment 1 at the Burton Russell Swine Research Farm, United Feeds, Inc., Frankfort, IN.

A total of 1760 crossbred pigs (Bunge Line 5 × Bunge Line 13) were weaned at 17 ± .2 d of age and allotted to treatment 22 h post-weaning. Pigs were randomly allotted to treatment pens on the basis of gender and weight.

The same dietary regimen as in Experiment 1 was followed (Table 2). The same management protocol as described for Experiment 1 was employed in this experiment. Pigs in all treatments were given the same floor-space allowance (.17 m^2/pig). In large groups, feeders were positioned in either the center of the pen for the single-location design or in five different places for the multiple-location design (Figures 1 and 2). For the small-group-size pen, the feeder was positioned in the center of the pen.

Pig performance data were analyzed using the GLM procedure of SAS (1990). Pen was considered the experimental unit. The model included effects of treatment, trial, and replicate nested within trial. To test the effect of feeder location on feed disappearance the feeder was

considered the experimental unit. The model used to evaluate effects of feeder location on feed intake within the two large-group-size pen designs included effects of feeder location, trial, and replicate nested within trial.

Results and Discussion. Pig weight, variation in weight (as indicated by the coefficient of variation for each weigh period), and growth performance data are given in Table 5. There was an effect of trial on pig start weight, but, no interaction between trial and treatment. Feed-disappearance data are given in Table 6.

Pigs in large groups compared to those in the small groups had similar live weight at the end of wk 1, but lower weight at the end of wk 4 (Table 5). Variation in live weight was not affected by pen design. Pigs in both of the large group treatments compared to the pigs in the small groups had lower ADG and ADFI for wk 1 through 4 and for wk 2 through 4 (Table 5). Pen design did not affect G:F. Morbidity and mortality were less than 1% in all treatment groups.

Similarly, Morrow and Walker (1994) found no difference in growth performance or feed intake between pigs kept in pens of 20 animals with single-spaced feeders placed either side-by-side or apart. These authors also found no affect of feeder placement on either queuing behavior or number of enforced withdrawals from the feeder in pigs kept in groups of 20. Those results, together with the results of the current study, suggest that feeders positioned in multiple locations within a pen do not result in higher levels of feed intake compared to feeders positioned together at a single location.

Feed disappearance was similar for all feeder locations in both large-group pen designs (Table 6). However, in the large group, multiple feeder locations pens, feed disappearance during wk 1 tended to be higher for feeder location M-2 (Table 6, Figure 2). For wk 2 through 4, feed disappearance tended to be numerically lower for feeder location M-5 in large group, multiple feeder locations pens (Table 6, Figure 2).

Social facilitation of feeding behavior has been reported in early weaned piglets, i.e., piglets were stimulated to feed when they observed penmates doing so (Wood-Gush and Csermely, 1981), and may explain, in part, the tendency here for pigs in large-group-size, multiple feeder locations pens to consume more feed from a single location (viz., location M-2) compared to other locations in the pen. In addition, other pen features, such as resting, drinking, and excretion areas have been shown to influence the pigs' preference for a feeding location (Petherick, 1983). Additional research is needed to understand the feeding behavior of pigs placed in large groups and/or allowed different feeder placements within a pen.

Implications

Results of this experiment indicate that penning weanling pigs in groups of 100 compared to groups of 20 pigs lowers weight gain and increases variation in body weight, and that reducing floor space by removing half of calculated "free space" reduces piglet weight gain. However, the similar growth performance observed between large and small group sizes at reduced floor-space

allowance in this experiment supports the hypothesis that the required floor-space allowance for maximum growth rate may decrease with increasing group size. Morever, within groups of 100 pigs, placing several multiple-space feeders together at a single location compared to placing the feeders in multiple locations doesn't improve pig performance.

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Figure 1. Large-pen design with single feeder location (Experiment 1 and 2)

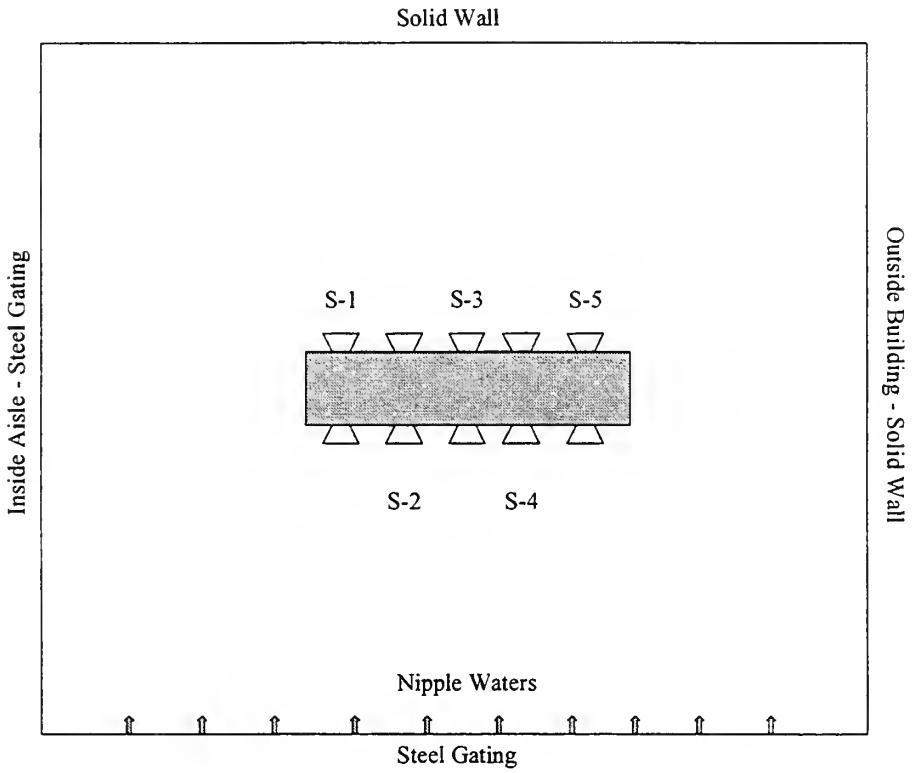
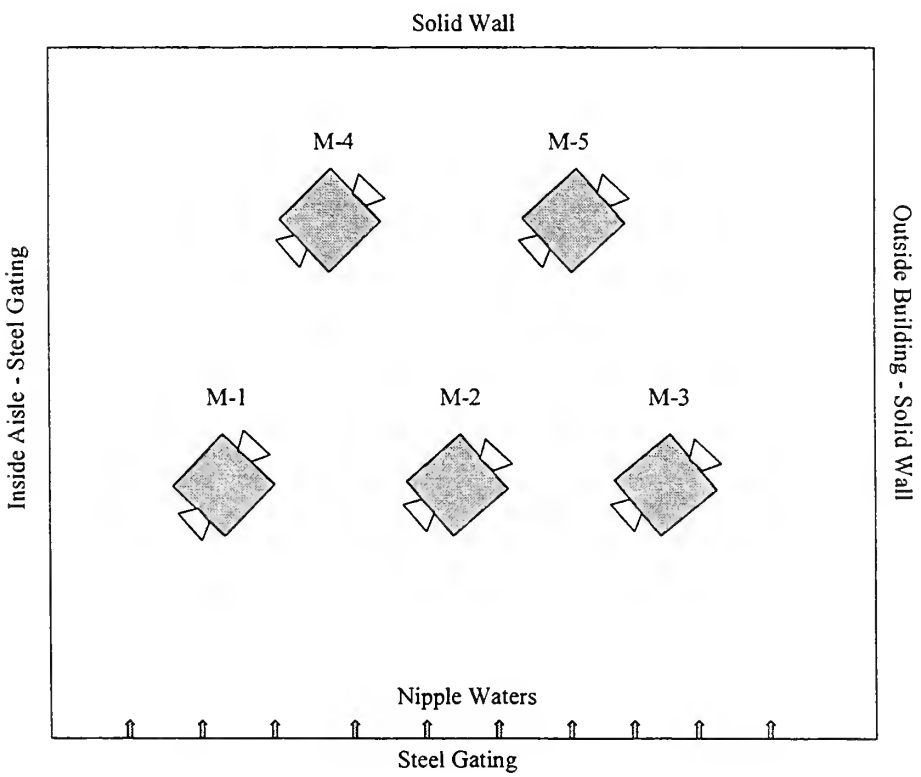


Figure 2. Large-pen design with multiple-feeder locations (Experiment 2)



Management of the Nursery Pig[1]

Ioannis Mavromichalis

Management is the key element that brings together genetics, facilities, and nutrition to achieve maximal performance in nursery pigs (3 to 25 kg of body weight). The ultimate goals of a any commercial nursery program should be to (1) maximize performance, in terms of growth and feed efficiency, and at the same time, (2) to minimize losses due to mortality and morbidity. Achievement of these goals requires a tremendous amount of resources during the delicate phase of postweaning growth.

SETTING UP THE STAGE

Nursery facilities, environmental control units, and equipment need to be properly prepared before the delivery of weaned pigs. When pigs arrive, they must find a place that is clean, warm, and draft-free with equipment (e.g., heaters, fans, lights, feeders, waterers, gates, etc.) already checked and adjusted for proper use.

Facilities

Preparing nursery facilities begins with thoroughly cleaning and disinfecting all rooms and equipment. The importance of the simple procedure of cleaning and sanitizing can not be emphasized enough! Ideally, pigs should be weaned in off-site nursery facilities that operate on an all-in/all-out by building or even better by site, management scheme. However, even in continuous-flow nurseries, meticulous cleaning and disinfecting of rooms or individual pens pays dividends in terms of enhanced growth performance. Today, it is well established that when pigs are placed in dirty nursery facilities, they grow slower and require more nutrient-dense, and thus more expensive, diets than pigs that are housed in properly sanitized facilities.

Timing of the cleaning procedure is important. Clean up must start as soon as possible after the last group of pigs is removed, because feed, dust, and fecal material tend to dry-up pretty quickly. Also, soaking for a few hours increases the effectiveness of pressure washing in removing dirt. It is important to remember that residual organic matter reduces greatly the potency of disinfecting agents. Paying attention to such details as frequent emptying of manure pits, cleaning under the slats, dusting-off fan blades, air ducts and light bulbs, and changing boot-cleaning solutions increases the level of hygiene and reduces the concentration of environmental pathogens to which pigs ultimately will be exposed.

Among other tasks that are not related to nursery rooms but they must be completed before pigs arrive include:

1. Repair and maintenance of exterior areas, and support buildings and equipment.
2. Replenishment of feed silos, which might contain late-nursery feed, with starter diets.
3. Rodent- and other wildlife-control.
4. Cleansing of coveralls, boots, pallets, carts, scales, scoops, etc.

[1] Adapted from a series of articles that appeared in *Pig Progress* magazine in 1999 by Ioannis Mavromichalis

Again, the importance of cleaning can not be overemphasized. It should be kept in mind that newcoming pigs (2 to 4 weeks of age) lack the robust immune system of a well-established older pig. At this age, pigs are extremely vulnerable to pathogenic microorganisms and thus, every effort should be made to reduce the concentration and transmission of these microorganisms from the environment.

Setting controls

Recently weaned pigs are not able to regulate effectively their body temperature because natural insulation (fat and pelage) and feed intake are not adequately developed at this age. Weaned pigs of about 5-kg body weight require a temperature of 30 to 32° C at animal level during the first week postweaning. Factors like facility design, floor material, lying mats, air circulation, stocking density, bedding, and feed intake affect the amount of supplemental heat required for maximal performance. In any case, the desired room temperature should be reached before pigs arrive. A thermometer probe that is placed on animal level helps in monitoring temperature but this does not reflect accurately animal comfort. Nevertheless, it is quite difficult to heat-stress weaned pigs and thus, it is suggested to start with a setting for upper recommended temperature and progressively adjust it downward based on the lying behavior of the animals.

Air circulation, also, should be monitored closely during the first weak after weaning. For recently weaned pigs, an air renewal rate of 0.1 m^2/hour per kg of body weight usually is more than enough, because excessive ventilation reduces air temperature without increasing air quality at this stage. An air speed across the bodies of pigs of more than 0.1 m/sec should be avoided because drafts decrease animal comfort and increase requirements for supplemental heat. An air speed of 0.3 m/sec constitutes a draft. In pens with slatted floors it might be difficult to achieve optimal temperature at animal level because drafts are easy to develop. In this case, a solid lying mat (rubber or plywood) can increase animal comfort and help in establishing lying and dunging areas. Pigs are not affected by relative humidity as long as it ranges between 60 and 80%.

Adjusting pen equipment

In each pen, feeders and watering devices are two pieces of equipment that need special attention before pigs move in. Feeders must be cleaned and disinfected along with the rest of the facilities and equipment. Moreover, adjustment of the height or opening of feed gates is required. The best strategy to adjust an empty and clean feeder is to shut down the feed gate/opening and then fill the feeder with feed. By operating slowly the adjustment mechanism a small stream of feed must be allowed to flow through the opening to cover approximately one-third of the feeder pan. If the diet is fed in meal form then it is highly possible that it might bridge easily. Therefore, feeders must be rechecked after pigs start consuming feed.

It is recommended to fill feeders shortly after pigs arrive to stimulate their interest and avoid stale feed. No more than a day's worth of feed should be placed in feeders during the very first days after weaning. Actually, offering feed two or even three times per day might increase feed intake in some occasions. Offering feed on a floor mat might also encourage pigs to eat but feed wastage could be excessive without a rim.

Waterers, also, should be carefully inspected in each and every pen because water consumption is even more important than feed intake during the first hours postweaning. If water cups are used, it is advisable to allow a small quantity of clean water to fill them right before or after pigs are placed in pens. This is especially helpful if pigs not used to drink from cups in farrowing houses. Nipple waterers must be adjusted at shoulder-height based on the size of the average pig for each pen. It is recommended to let water trickle from nipples during the first couple of days or until pigs become familiar drinking from these devices.

If bedding is used then, it should be piled close to the assigned lying area and be allowed to reach room temperature before pigs arrive. Wet bedding should never be used. Other pen devices such as chains, toys, special feed and water dispensers must be cleaned and properly adjusted before placed in pens. Finally, all pen gates and locks must be checked and secured in place.

When pigs arrive

In most units, pigs will arrive during morning or early afternoon hours. Weaning during evening hours has been shown to reduce aggression among pigs but it might be difficult to arrange for labor in late hours. In any case, it is a good idea to allow pigs to suckle a last meal from their mothers before weaning. This could help them to cope better during the first hours postweaning. If pigs are transported over long distance it might be helpful to have ready an electrolyte and (or) glucose solution for severely dehydrated pigs.

Pigs should be sorted by weight and immediately placed in their assigned pens. During sorting and moving the room temperature can drop dramatically unless close attention is paid to avoid open doors. Any treatments (i.e., vaccinations, ear-tagging, etc.) must be performed at the time of unloading to avoid double handling of the pigs. Giving certain vaccinations at weaning might reduce feed intake during the first weak postweaning and therefore, it is recommended often to avoid such treatments if possible.

During the next few hours, after pigs are placed in the nursery, it is important to observe them closely to make sure that they become familiar with their surroundings, feeders, and waterers. Fighting might be excessive especially in pens where pigs are not from the same litter. Although penning littermates together reduces aggression, sorting by size results in increased growth performance because pigs are generally more active. Any pig that is seriously injured should be removed and treated promptly in a "hospital" pen.

FEED MANAGEMENT

Development of farm-specific feeding programs for nursery pigs involves the establishment of (1) dietary specifications, (2) number and time of diet changes, and (3) amount of each diet to be fed per pig. A combination of high quality diets and a proper feed management plan is required to prevent overfeeding of expensive diets without sacrificing performance. A phase-feeding program that employs complex and simple starter diets prevents postweaning growth depression by adjusting pigs rapidly but smoothly to low cost diets at a pace that coincides with digestive system maturation.

Diet specifications

The variable that influences most dietary specifications of nursery diets, in terms of nutrient and ingredient concentrations, is body weight (Table 1). As body weight increases, pigs require diets of lesser complexity (ingredients) and density (nutrients). Other factors that affect dietary specifications, but a lesser magnitude, are weaning age, health status, genetic potential, and farm expertise. These factors, however, affect growth rates as well and thus, body weight at a certain age becomes hard to predict!

Quite often, producers exercise little or no control over specifications of diets they use in their nurseries. For a number of good reasons, they tend to rely on commercial manufacturers for quality nursery diets. First, there is a high degree of expertise associated with designing and manufacturing nursery diets; especially those designed for postweaned pigs. Secondly, nursery diets require a large inventory of expensive ingredients that tend to deteriorate rapidly, particularly during hot weather. Thirdly, these diets that are used in relatively small amounts often are pelleted or thermally processed. Therefore, mixing of nursery diets at farm-level becomes an expensive undertaking, particularly on a small-scale basis.

The number of diets used in commercial nursery programs is highly variable and hard to categorize. It is not uncommon to find programs with seven diets for pigs weaned around 12 days-of-age and others where only one diet is fed throughout the whole nursery period! However, four diets are though to be enough to cover any detailed feeding program (Table 2). As a general rule, four diets are recommended for segregated early weaned pigs, which are weaned in off-site nurseries between 12 and 17 days-of age. Three diets are sufficient for pigs weaned around three weeks of age, whereas no more than two diets are required for pigs weaned beyond four weeks-of-age. When only one diet must be fed throughout the whole nursery period it is recommended to use a diet of medium complexity to accomplish a balance across performance and cost.

Based on actual animal performance, it might be beneficial to use dietary specifications that are higher or lower than those depicted in Table 1. The first diet postweaning (starter) must be highly dense in nutrients and complex in terms of special ingredients. The second diet (link) should be characterized by a rather dense nutrient profile but special ingredient concentrations should decrease to allow pigs to adjust to lower cost ingredients. The rest of the nursery diets must be quite simple and inexpensive. The very last diet often is nothing but a fortified corn-soybean meal-type mix.

Diet changes

The decision when to switch from complex to simpler diets is a difficult one! Undoubtedly, complex diets are able to support maximal growth performance but there is a certain point after which they offer no benefits compared to less fortified diets. It is this specific point of time that needs to be defined accurately so that neither overfeeding of expensive diets nor performance loss occurs.

The optimal criterion for establishing diet change sequences is body weight of pigs because diets are formulated to meet nutrient requirements for a specific range of body weight. This practice, however, requires frequent weighing of a representative sample of pigs for each batch, which

should be large enough to account for natural variability in live weight. An alternative approach would be to establish farm-specific growth curves over time (Figure 1). For this, a relatively large number of pigs must be closely followed throughout the entire nursery period with weekly body weights recorded. Growth performance, however, is highly variable during the nursery phase even when genetics and health remain relatively standard among groups of pigs. Therefore, a representative growth curve needs to be based on several batches of pigs of the same health status and genetic background. Even then, seasonal curves might be more appropriate instead of a single year-round reference curve. It is obvious that where pigs are brought in from several undisclosed sources, development of a standard growth curve is almost impractical!

A more practical feed management plan bases diet changes on time. This practice, however, assumes also a relatively well-established pattern of growth that allows diets to be assigned over time (Table 3). Time-based feed programs require a low level of management and are easy to implement and follow. Their greatest disadvantage, however, is that small pigs do not receive enough of the complex diets and thus, their performance is not maximized, whereas large pigs receive more than they actually need to thrive. This method is best for small batches of pigs, but as batch size increases it becomes quite expensive not to adjust feeding methods to account for weaning weight variability.

Feed allowance plan

The average weaning weight (wt) and its standard deviation (sd) are easily calculated in any farm. Although average weight is quite variable, because it depends on weaning age and management, standard deviation has been calculated to be less variable (around 1 kg). This information can be used to calculate the number of pigs that are expected to be falling in different weight groups around the average (Table 3). For example, in a 1,000 batch of weaners with an average weaning weight of 6 kg and a standard deviation of 1 kg, only 68% of the pigs are expected to be between 5 and 7 kg (wt ± sd), whereas 16% of the pigs are expected to be less than 5 kg and another 16% above 7 kg. Table 3 can be used to decide how many and which weight groups to establish based on total number of pigs per batch and available facilities. As a rule of thumb, the larger the batches of pigs and the smaller the number of pigs per pen, the more the weight categories that can be established. Each group, then, can be fed according to a predetermined feed allowance schedule. A three-phase feed allowance guide for 3-week old weaners is presented in Table 4.

A feed management plan for the above group of 1,000 weaners is calculated in Table 5. First, weight groups that correspond to a 6-kg average weaning weight are copied from Table 3 to column A in Table 5. Then, the number of pigs falling in each group is calculated in column B by multiplying percentages from Table 3 with 1,000 (number of pigs in batch). Then, a decision is made to establish four working weight groups (column C). One for light (< 4 kg), one for heavy (> 7 kg), and two for medium (4 to 5 and 5 to 6) weight pigs. The two medium groups could be merged in only one large group, but the size of this batch is large enough to justify management of these two sizable groups. This step is rather subjective and depends on how many separate weight groups can be handled successfully at each farm. In column D, the total number of pigs for each working weigh group is summed up. Column E is basically pasted from Table 4 and these figures are used to calculate total feed allowance by group in column F. If the

size of feed bags is known (here is set to 25 kg) then the number of bags needed for each group can be determined easily (column G). In the same worksheet, the number of pens required for each group (column H) can be calculated by dividing the number of pigs in each group by the desired pen density (here is set to 20 pigs/pen). Pen density could be adjusted based on weight of each group. Finally, the amount of feed that need to be delivered in each pen (column I) is calculated by dividing the total feed per group (column F) by the number of pens per group (column H).

As soon as pigs arrive at the nursery site, they should be placed in their already assigned pens according to body weight and allowed to consume their predetermined feed allowance before introducing the next diet. Afterwards, all pigs should be given equal amounts of a link and grower diet based on recommended allowances from Table 4. Of course, the above example should be modified to accommodate for variation in weaning age and farm-specific recommendations of feed allowances.

EARLY FEED INTAKE

Low feed intake has always been a problem in nursery pig management. It limits growth, increases temperature requirements, intensifies morbidity and mortality, and reduces turn over of expensive nursery facilities. Modern nursery pigs have a tremendous genetic potential for lean growth but they achieve approximately 70% of it under most commercial nursery facilities. Basically, pigs weaned at about 2 to 3 weeks-of-age are constantly in an energy-deficient phase in which feed intake determines growth performance. For every 100 g of extra feed per day that weaned pigs ingest during the first week postweaning, body weight increases by 1,500 g at the end of the fourth week postweaning. However, most pigs experience severe anorexia immediately following weaning and as a consequence, their appetites and growth rates remain depressed for the duration of the remaining nursery period. It is not uncommon for large numbers of pigs to starve for as long as 3 to 5 days after weaning. This has dramatic effects on overall performance during the growing-finishing period. Pigs that barely maintain their weaning weight during the first week postweaning may take an extra 10 to 20 days to reach market weight compared to pigs that grow at their preweaning gain rates during the same period. Early weaned pigs require 350 to 400 g of dry feed per day, during the critical first week postweaning, to maintain their pre-weaning growth rate of approximately 350 g per day. Actual feed intakes, however, rarely exceed 200 to 250 g per day during this period!

The reasons for postweaning anorexia are many and complex. The change in the feed form, from liquid milk to dry feed, might explain the difficulty with which baby pigs associate dry feed with nourishment. Several antinutritional factors in certain feed ingredients are also singled out as causes of low feed intakes. Social and environmental stress factors are also held responsible for poor feed intakes and growth rates. Certainly, there is a plethora of good reasons for a recently weaned pig to go off feed after weaning. Nursery managers, however, are fortunate to have an array of measures to prevent starvation and even achieve feed intake levels close to maximal, but always with a cost!

Milk replacers. Nursery pigs will readily consume a liquid milk replacer. Feeding a liquid milk replacer during the first 3 to 4 days postweaning compared to a pelleted starter formula can actually double dry matter intake. However, pigs reared solely on a liquid diet may as well fail

to relate to dry feed unless the milk replacer is combined it with a high quality starter diet. Thick, high-protein milk replacers support maximal growth rates. Good sanitation and frequent feeding are essential to prevent spoilage and attract pigs to eat. Milk replacers are best suited for low-weight and orphan pigs because of cost constrains.

Liquid diets. Offering a soup of water (or a liquid dairy product) and dry feed can increase dramatically (up to 300%) the intake of dry matter during the first week postweaning. Although a ratio close to 3:1 water to dry feed is usually recommended for most automatic pipeline feeding systems, nursery pigs can utilize efficiently even more dilute mixtures (up to 5:1). Such blends, however, result in excessive effluent output without any advantages in terms of growth performance. A recent advance in liquid feeding involves fermentation of the soup to promote growth of lactic acid-producing bacteria, with beneficial effects on feed intake, growth rates, and intestinal health. Liquid feeding of weaners is not a widespread practice, however promising. A lack of scientific knowledge on the area of liquid nutrition, combined with the inadequacy of most current feeding systems, prevent most producers from adopting it. Fortunately, the scientific community and equipment manufacturers are actively engaged in materializing this concept.

Floor feeding. Spreading a small quantity of feed on the floor appears to encourage pigs to rut and ingest it. In slatted pens; a floorboard with a rim or a large plastic tray, securely attached to the floor, can be used. A mash provides better results over pellets as pigs roll and push pellets instead of picking them up. Spreading the feed or placing the board near the feeder seems to encourage pigs further to consume more feed from the feeder. Pigs require only 2 to 3 days of floor feeding before they become accustomed eating from regular feeders. Frequent feeding stimulates pigs to eat more and prevents wastage of uneaten portions. Actually, there are commercial contraptions designed to release small amounts of feed in frequent intervals, with considerable savings in labor and feed. Growth performance comparable to preweaning rates can be easily achieved, but wastage can become excessive without proper management of floor feeding.

Special ingredients. Several special ingredients and feed additives enhance growth performance. Milk products (i.e., whey, lactose, and skim milk) offer a source of highly digestible sugars and proteins and thus, they have become an integral part of most nursery diets. Spray-dried animal plasma (3-7% of diet) and zinc oxide (0.27-0.42% of diet), which appear to work best during the first 2 to 3 weeks postweaning, increase growth performance by about 30 and 15%, respectively. Cupric sulfate (0.08% of diet) provides comparable results at a lower cost during the late-nursery period. Antibiotics, probably the most potent additive in promoting nursery pig performance, are gradually phased-out. However, organic acids (e.g., lactate and fumarate, 1-3% of diet) and perhaps some oligosaccharides appear to be most promising alternatives to antibiotics, presently. A plethora of other feed additives (e.g., flavors, herbs and spices, yeasts, carnitine, vitamin C, excesses of vitamins B and E, organic minerals, chromium) are often used to augment starter diets. However, their use is rather questionable and circumstantial because of lack of solid scientific evidence to justify their functions.

Gruel feeding. In farms where pigs are fed dry diets on a regular basis, a warm gruel (50:50) of feed and water (or a liquid milk co-product) can be offered to weaned pigs in special bowl-type

feeders during the first 2 to 3 days postweaning. This practice may prevent starvation, but still some piglets fail to adapt to dry feed, unless the gruel is gradually thickened. Results with this practice have been variable because of labor (frequent mixing and feeding) and sanitation (feed spoilage) requirements.

THE FIRST WEEK POSTWEANING

The first week postweaning is the period that calls for the most attention in a growing pig's life because it is then when most problems arise. Although, the successful establishment of recently weaned pigs requires a high degree of dedication and zeal from the nursery manager, it pays up during the growing-finishing period because pigs grow more efficiently, are less prone to diseases and thus, they become much easier to manage. The three areas of management that need daily attention during the first week postweaning are (1) health and well being, (2) environmental conditions, and (3) feed intake. The proper attitude in managing early-weaned pigs to recognize that they are inexperienced, immature, and highly vulnerable newborns that require more care and attention than finishing pigs!

Health concerns

Pigs that are weaned at about two to three weeks of age are characterized by a very weak immune system, in terms of both passive (sow's milk) and active (self) protection against pathogenic agents. A high level of biosecurity is the best line of attack to ensure exclusion and elimination of most pathogenic microorganisms. Health also can deteriorate easily from improper management, poor environmental conditions, and inadequate feed consumption. Pigs that are cold and (or) starve during the first couple days following weaning are especially vulnerable to pathogens.

Signs of deteriorating health include sneezing, coughing, rough hair, and lack of activity. Activity is an important indicator of overall health. Pigs that are lethargic, or pile up in a corner indicate discomfort and lack of well being. Pigs that fall behind in growth are usually more susceptible to succumb to diseases or become chronic carriers of them. If poor-doing pigs are left unattended they develop usually into runt pigs that are extremely difficult to recover. Runt pigs are the slowest and most problematic pigs in a herd and their rearing seldom is profitable. In some management schemes, runt pigs are euthanized routinely to avoid a possible disease outbreak and avoid profit loss. On the other hand, timely identification and isolation of poor doing pigs might be a cost-effective alternative in some enterprises. A designated hospital pen, which is usually located in the most warm and draft-free area of the nursery, may help in providing some extra care and individual attention to pigs that fail to thrive. The decision to keep or destroy poor-doing pigs depends on pig prices, labor availability and expertise, and size of the operation. Either way, the most important aspect is to identify and remove these pigs before they deteriorate any further or start affecting other pigs.

A special area of attention, especially during the first two-three days postweaning is fighting among penmates. Even with littermates penned together some fighting will take place, especially if litters are not kept intact. In pens filled with unfamiliar pigs, fighting is a natural process for the establishment of a new hierarchy. However, in some cases the lowest ranking pig(s) might be so severely debilitated that human intervention is needed. Fighting is more intense among pigs of comparable size and becomes less of a problem as pig weight spread

increases. Fighting is less intense in dim lighting and when pigs are weaned during the evening hours. Inadequate lying and feeding space is a common cause of aggression among pigs.

Environmental conditions

Although many textbooks define the optimum environmental conditions for nursery pigs, animal behavior remains the ultimate indicator of comfort and welfare. Pigs that are cold, sick, or try to avoid drafts tend to mingle. On the contrary, when temperature exceeds the upper limit of their zone of comfort, pigs spread out as much as they can. Panting is a sign that the room temperature is way too high! It must be remembered thought that recently weaned pigs require approximately 30 to 32 °C during the first week postweaning and therefore, they are more likely to be cold than hot. Factors such as floor material, bedding, and pen divisions influence greatly the optimum environmental conditions. Of course, a minimum ventilation rate should be maintained throughout the first week postweaning.

Apart from temperature and ventilation controls, other checkpoints are (1) heaters and fans for proper function and obstructions, (2) level of manure in pits and ammonia in the house, (3) light bulbs and lighting schedule, (4) rodent presence and control, (5) feed delivery system for proper function and blockage including outside bins, and (5) presence of excessive moisture in walls and equipment. At the pen level, a daily inspection schedule must include (1) feeders for feed level and bridging, (2) waterers for blockage, pressure, and fouling in cup drinkers, (3) bedding material, if any, for excessive moisture and fouling, (4) pen gates for unsecured latches, (5) laying and defecation areas for proper establishment, cleanliness, and (6) prevalence of diarrhea.

Feed and feeders

During the first week postweaning, pigs face a dramatic change in their feeding regimen. Not only they have to switch from milk to dry feed but they also have to associate the feeder and its contents to the feeding process without any guidance or stimulation. Pigs that fail to identify feed and (or) consume enough quantities of it immediately following weaning require a higher level of management input and become susceptible to diseases. Therefore, quick establishment of a vigorous feeding behavior after weaning is of paramount importance for both pigs and managers.

Pigs appear to recognize and readily consume feed spread on the floor or on a sleeping mat. Therefore it is common to offer a limited quantity of feed in such a way during the first two to three days postweaning. However, it is highly desirable for pigs to start quickly consuming feed from feeders to prevent feed wastage and reduce labor associated with floor feeding. Signs that pigs have started eating are (1) wet feed in trough walls, (2) dark feces (milk feces are yellowish-brown), (3) expanded, round abdomens, and (4) calm and contented pigs. Pigs that have been starving for two to four days are easily identified by their (1) rough and sometimes dehydrated appearance, (2) shrunk abdomens, (3) protruding backbone and ribs, and (4) lethargic and exhausted condition.

Other points of a proper management checklist should include feeder adjustment to prevent wastage and bridging. Approximately, only one third of the feeder floor must be covered with a thin layer of feed to prevent pigs from wasting it. Without proper adjustment, it not uncommon for feed wastage to range from 7 to 20%! On the other hand, feed bridging might become also a

serious problem, especially with meal diets. Accumulation of wetted feed on feeder walls, gate, and floor might proceed to the point that feed flow is drastically reduced or completely ceases. Feed flowability can also change from batch to batch, especially in the case of meal-type diets, because of marked changes in formulation. Even within the same batch of feed, flow problems may arise with diets that contain high amounts of milk products during times of high environmental temperature and humidity. Overall, it is recommended to inspect, clean, and adjust feeders as often as needed during the first week postweaning to ensure continuous feed flow and eliminate wastage.

CLOSING REMARKS

Managing a nursery facility is a challenging task. It requires attention to detail and a lot of patience. It should be kept in mind that, today, weaned pigs are extremely young, vulnerable, and inexperienced. Going the extra mile to ensure that pigs are received in a comfortable and healthy environment is the only reassurance toward fast-growing grower pigs.

Table 1 Specifications of a phase-feeding program for nursery pigs

Item	Diet 1 (3 to 5 kg)	Diet 2 (5 to 8 kg)	Diet 3 (8 to 15 kg)	Diet 4 (15 to 25 kg)
Ingredients, %				
Corn/wheat/groats	no limit	no limit	no limit	no limit
Barley/oats/rye	0	0	0 – 15	0 – 30
Soybean meal	0 – 10	10 – 25	no limit	no limit
Soybean proteins	0 – 10	0 – 5	0	0
Dried whey	25 – 35	15 – 25	5 – 15	0 – 5
Plasma protein	6 – 8	4 – 6	0 – 2	0
Fish meal	0 – 8	0 – 8	0 – 5	0 – 5
Fat or oil	3 – 6	3 – 6	0 – 6	0 – 6
Salt	0.5	0.5	0.4	0.3
Growth promotants	++	+	+	(?)
Nutrients				
Digestible energy, kcal/kg	3,600	3,600	3,600	3,600
Protein, %	25 – 27	23 – 26	20 – 24	18 – 22
True ileal digestible lysine, %	1.7 – 1.8	1.5 – 1.7	1.3 – 1.5	1.1 – 1.3
Calcium, %	0.9 – 1.0	0.8 – 0.9	0.8 – 0.9	0.7 – 0.8
Available phosphorus, %	0.5 – 0.6	0.4 – 0.5	0.4 – 0.5	0.3 – 0.4
Zinc (from ZnO), mg/kg	3,000	3,000	2,000	-
Copper (from $CuSO_4$), mg/kg	-	-	-	150 – 250
Lactose, %	25 – 35	20 – 30	5 – 15	0 – 5
Pelleted	Yes	Yes	Yes/No	No

Table 2 Selection of nursery diets according to weaning age

System	Age (days)	Diet 1	Diet 2	Diet 3	Diet 4
Segregated early weaning (SEW)	12 to 17	√	√	√	√
Early weaning (EW)	18 to 23		√	√	√
Conventional weaning (CW)	24 to 34		√		√
Late weaning (LW)	> 35			√	√

Table 3 Distribution of weaning weights with a standard deviation of 1.0 kg

Weight (kg)	Average weaning weight (kg)				
	4.0	5.0	6.0	7.0	8.0
	Percentage of pigs falling within each weight group				
< 2	2.3				
2 – 3	13.6	2.3			
3 – 4	34.1	13.6	2.3		
4 – 5	34.1	34.1	13.6	2.3	
5 – 6	13.6	34.1	34.1	13.6	2.3
6 – 7	2.3	13.6	34.1	34.1	13.6
7 – 8		2.3	13.6	34.1	34.1
8 – 9			2.3	13.6	34.1
9 – 10				2.3	13.6
> 10					2.3

Table 4 Recommended feed allowances (kg) for early-weaned pigs

Diet	Average weaning weight (kg)				
	< 4	4 – 5	5 – 6	6 – 7	> 7
Starter (e.g. diet 2)	2.5	2.0	1.5	1.0	0.5
Link (e.g. diet 3)	8.0	8.0	8.0	8.0	8.0
Grower (e.g. diet 4)	16.0	16.0	16.0	16.0	16.0

Table 5 Example worksheet for a 1,000-head nursery feed management plan (starter diet)

Weight groups (kg)	**Pigs**	**Working weight groups**	**Pigs/group**	**Feed/pig (kg)**	**Feed/group (kg)**	**Bags/group (25 kg each)**	**Pens/group (20 pigs each)**	**Feed/pen (kg)**
A	B	C	D	E	F	G	H	I
< 4	23							
4 – 5	136	< 5	159	2.0	318	13	8	40
5 – 6	341	5 – 6	341	1.5	512	20	17	30
6 – 7	341	6 – 7	341	1.0	341	14	17	20
7 – 8	136	> 7	159	1.0	80	3	8	10
> 8	23							
Total	1,000		1,000		1,250	50	50	

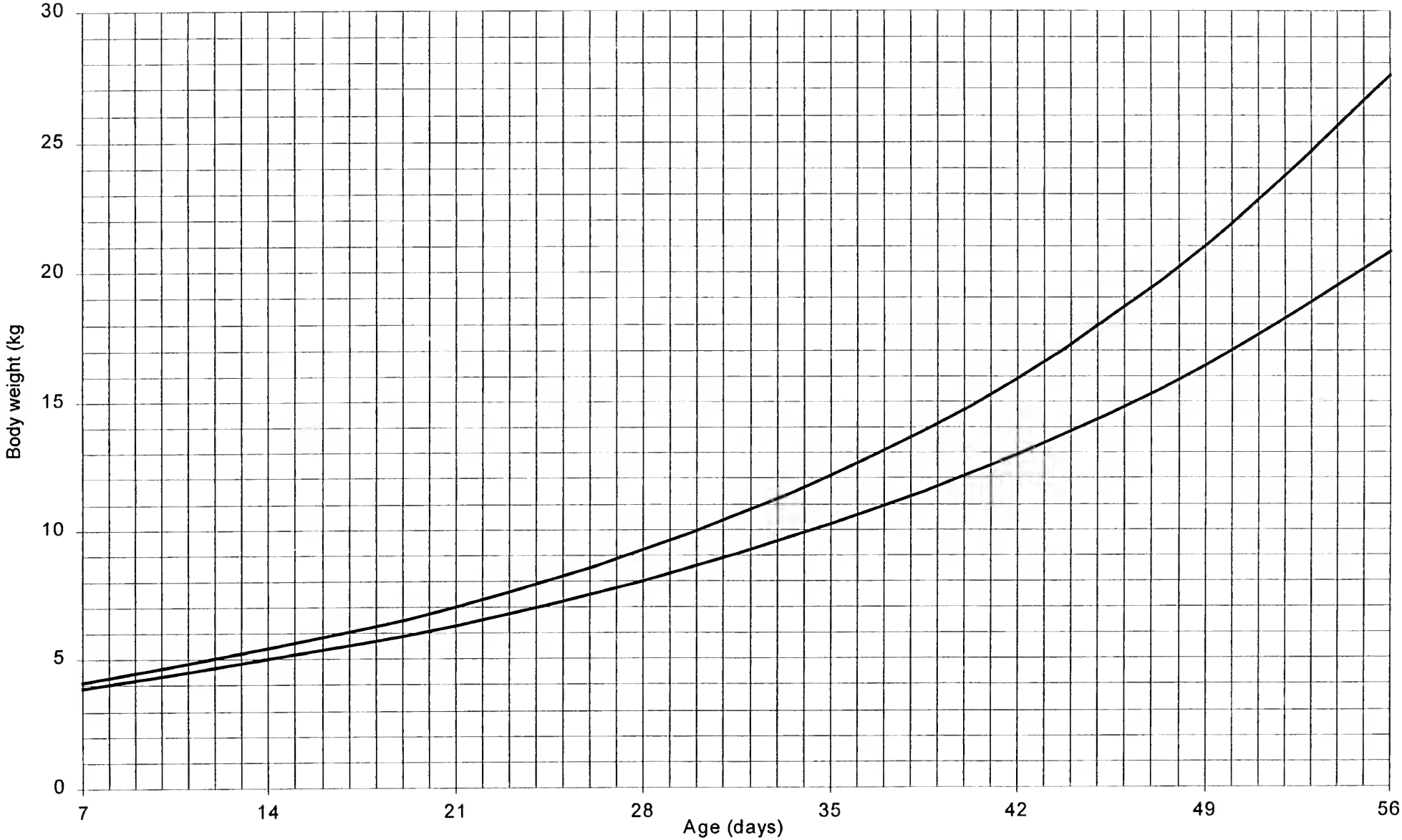


Figure 1. Example of a nursery pig growth curve over time. Note that postweaning growth depression is not depicted in this graph

Storage But Not Pelleting Destroys Lysine

Ioannis Mavromichalis and David H. Baker
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Complex diets are loaded with lysine and reducing sugars that can combine together and become worthless for pigs. Besides corn and soybean meal, major sources of lysine include animal plasma, whey, skim milk, fishmeal, and crystalline lysine. Lactose, the main reducing sugar in nursery diets, is furnished by milk products, and crystalline lactose itself. The SEW, Phase 1, and Phase 2 diets are the most vulnerable diets.

Although lysine is the most reactive nutrient, and often therefore serves as an indicator of quality deterioration, other nutrients are also susceptible. These include all free amino acids, and the vitamins thiamin and folacin. The losses incurred by the sugars themselves are usually negligible from a nutritional point of view.

The Maillard or non-enzymatic browning reaction that renders lysine unavailable is favored by high temperatures, humidity, time, and presence of trace minerals. Nursery diets are susceptible to lysine destruction during thermal processing (e.g., pelleting, expanding, extrusion) and during prolonged storage in warm and humid conditions. With most nursery diets being pelleted, some degree of lysine destruction is naturally expected. Also, many producers fill feeders with enough feed for 4 to 10 days to reduce labor and storage space, thus exposing costly diets to the warm and humid nursery environment for prolonged periods of time.

We examined the effects of pelleting and in-feeder storage on lysine bioavailability of a complex Phase 1 diet (28% lactose and 1.4% lysine). Surprisingly, pelleting did not reduce lysine bioavailability (Table 1). Being larger (5 mm) than most commercial pellets (2 to 4 mm), our pellets did not sustain any detectable lysine damage and thus, we are currently investigating smaller pellet sizes. On the other hand, placing the diet in metallic feeders in an occupied nursery facility for 1 week reduced lysine bioavailability by an average of 12% (Figure 1). The lysine loss was observed in both meal and pelleted feed.

To avoid losses of lysine and ensure maximal performance, we recommend more frequent feeding intervals, especially during the critical 2 weeks postweaning,. In addition, prolonged storage of starter diets during summer months is discouraged. Instead, fresh nursery diets should be prepared and fed immediately.

Table 1. Effects of storage and pelleting on lysine bioavailability of a complex nursery diet

Treatment	Lysine bioavailability, %
Meal	
Refrigerated	97
Exposed in nursery	88
Pellets	
Refrigerated	107
Exposed in nursery	92

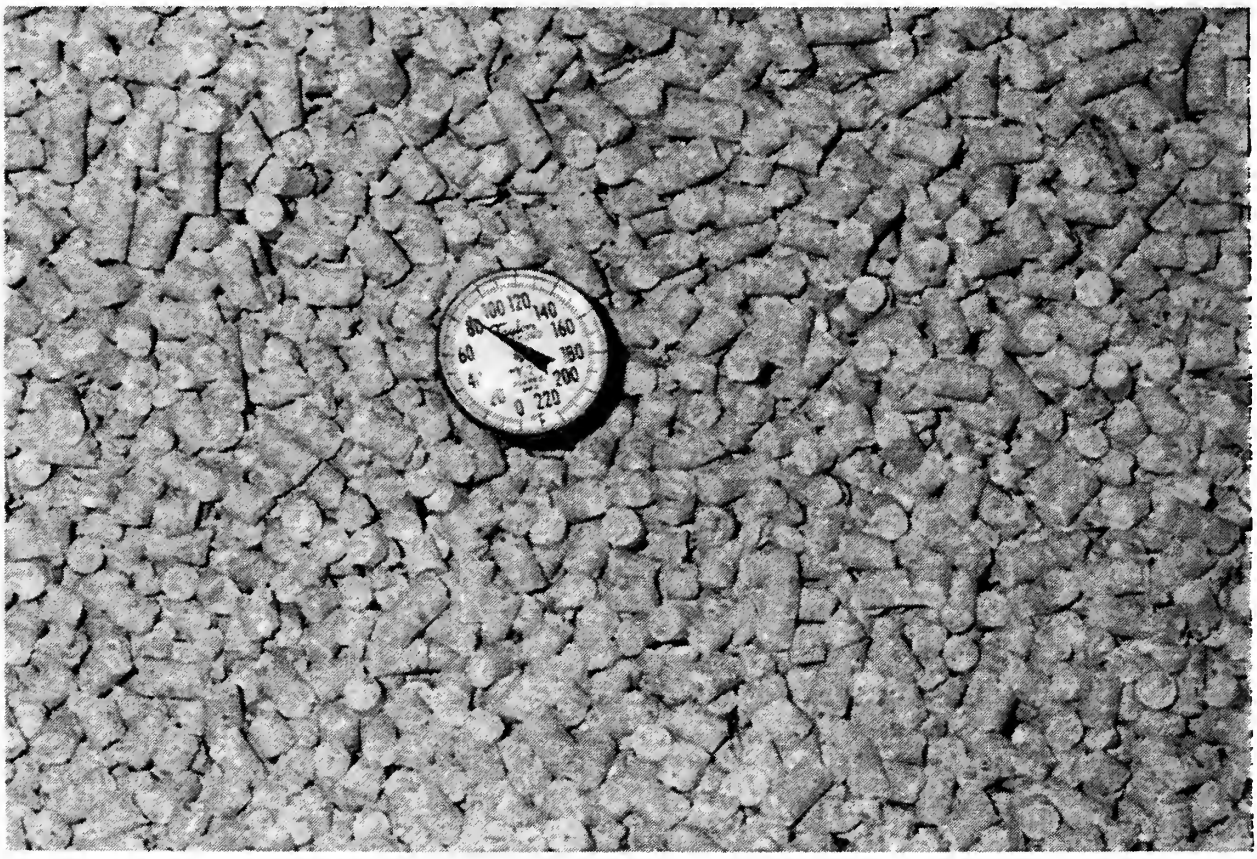

Figure 1. Exposing a complex diet in an occupied nursery facility for 1 week reduced lysine bioavailability by 12%.

Natural Feeding Enhances Appetite of Newly-Weaned Pigs

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Starvation and depressed postweaning appetite guarantee life-long poor growth rates, increased mortality and morbidity, and intensive management. Nursery pigs have a tremendous genetic potential for lean growth. However, low feed intakes allow them to achieve only 60 to 70% of it under most commercial conditions, mainly because feed intake determines growth. Pigs that barely maintain their weaning weight during the first week postweaning may take an extra 10 to 20 days to reach market weight compared to pigs that grow at their preweaning gain rates during the same period. With a preweaning growth rate of 250 g per day, weaned pigs require 250 to 300 g of feed per day to only maintain this growth rate during the critical first week postweaning. Actual feed intakes, however, rarely exceed 200 g per day during the same period in that many pigs actually eat no feed for the first several days postweaning.

We were able to enhance postweaning growth rates and actually achieve feed intake levels close to maximum by allowing pigs to follow their natural instincts of exploring and rutting for feed. In two experiments, pigs were offered an ample amount of feed on a rubber floorboard with rims, in addition to feed in feeders. In the first experiment, weaned pigs (21 days old) that were offered a meal complex diet on floorboard grew 150% faster during the first week postweaning than pigs with access only to mash in feeders (Table 1). In a second experiment, pellets were available in feeders, whereas the same diet in meal or pellet form was offered on the floorboard. During the first 3 days postweaning (weaning at 21 days-of-age), combined feed intake (excluding wastage) reached 345 g per day in pigs offered meal but not pellets on floorboard (Figure 1). Surprisingly, pigs that had pellets on the floorboard did as poorly as pigs offered nothing on the floorboard. Overall (d 0 to 7 postweaning) feed intake was improved by 75% and growth rate by 50% in pigs given a complete diet in meal form on the floorboard (with pellets in the feeder) for only 3 days following weaning. We are currently investigating different regimens and diet combinations to determine the optimum duration and diet sequence for this practice.

Several other subjective observations in this research included less fighting and increased comfort in floor (or tray)-fed pigs. Placing the floorboard near the feeder appeared to encourage pigs to consume more feed from the feeder and to avoid defecating on the board. Frequent floor-feeding (at least three times daily) appeared to stimulate pigs to explore and ingest more feed from the feeder. Finally, having proper feeding equipment can greatly reduce feed wastage.

Table 1. Floor-feeding and early growth performance of nursery pigs

Treatment	Gain, g/d	Feed intake, g/d
Exp. 1 (d 0 to 7 postweaning)		
No feed on board	71	-
Meal on board	175	-
Exp. 2 (d 0 to 3 postweaning)		
No feed on board	31	85
Pellets on board	91	100
Meal on board	330	345

Diet sprinkled on floorboard in front of feeder for the first 7 d (Exp. 1) or first 3 d (Exp. 2) postweaning. Performance data are for the first 7 d (Exp. 1) and the first 3 d (Exp. 2) postweaning.

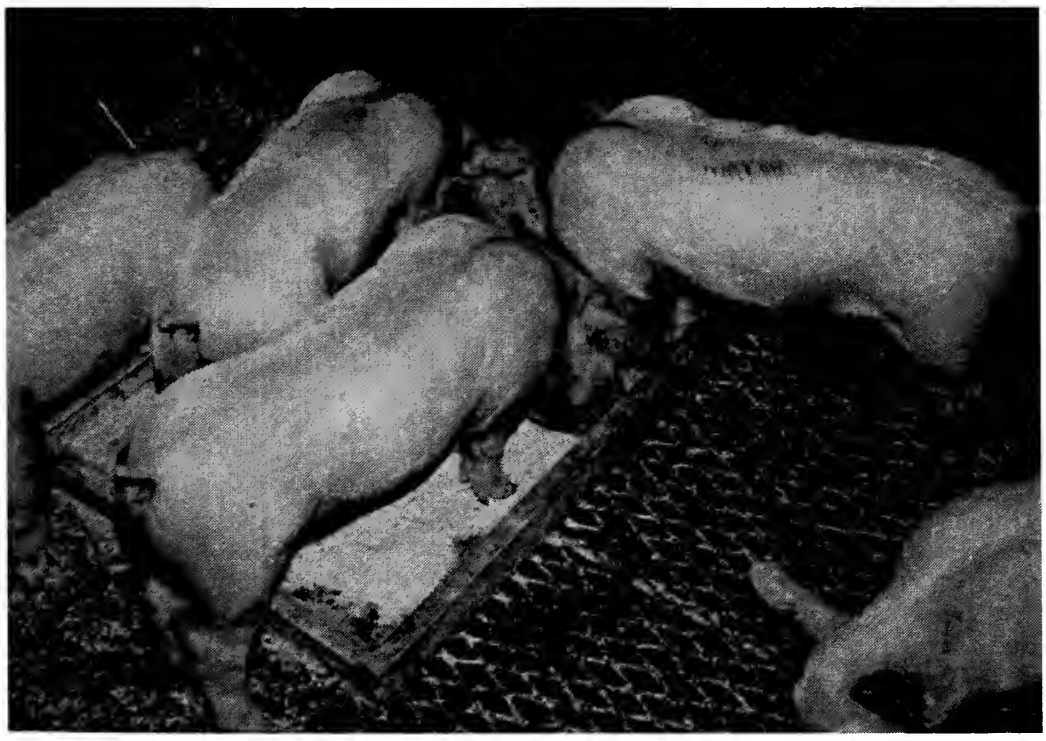

Figure 1. These pigs consumed 345 g of feed per day during the first 3 days after weaning at 21 days-of-age.

Side-Effects of Vaccinations in Nursery Pigs

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Vaccination regimens for nursery pigs require that injections be given at a time that will ensure maximal development of immunity. Protection against pathogens is of paramount importance in pigs weaned at or before 3 weeks because at this age, passive (i.e., antibodies from sow's milk) and active (i.e., antibodies produced by the pig itself) immunity are at their lowest points. Therefore, it is often recommended that the primary immunization be administered 1 to 2 weeks before weaning followed by a booster immunization 1 to 2 weeks after weaning.

Many producers prefer to administer vaccinations at the time of weaning to save on labor and time, and to decrease animal stress due to double handling. Immunity, however, develops at least 1 week after initial vaccination. Postponing vaccination until weaning, therefore, increases the risk of infection during the critical first week postweaning, especially in facilities of medium to low health status. Furthermore, mild local and systemic reactions to vaccination, which may depress growth performance after weaning, are expected because of the vigorous stimulation of the immune system following the initial vaccination.

Our research focused on the side effects of a vaccination regimen in which vaccinations were given 1 week before or at the time of weaning (21 days-of-age) on growth performance of pigs during the first week postweaning. Vaccinations given were 2 mL of Bordetella bronchiseptica-Erysipelothrix rhusiopathiae-Pasturella multocida bacterin-toxoid (Atrobac® 3; Pfizer, Exton, PA) and 2 mL of Mycoplasma hyopneumoniae bacterin (RespiSure®; Pfizer, Exton, PA). During the last week before weaning, pigs that were vaccinated grew as fast as pigs that were vaccinated at weaning (Table 1). Also, postweaning growth performance in terms of weight gain and total feed intake was not affected by either vaccination time. However, a depression in feed intake was detected during days 4 and 5 postweaning in pigs vaccinated at weaning (Figure 1).

Although overall growth performance was not affected by vaccinations at weaning, the 2-day drop in performance at days 4 and 5 postweaning (despite compensatory feed intakes on days 6 and 7) may be cause for concern.

Table 1. Side-effects of vaccinations in nursery pigs

Item	Vaccination time	
	Before weaning	At weaning
Preweaning (1 wk)		
Weight gain, g/d	190	184
Postweaning (1 wk)		
Weight gain, g/d	128	106
Feed intake, g/d	138	132
Gain:feed, g/kg	928	803

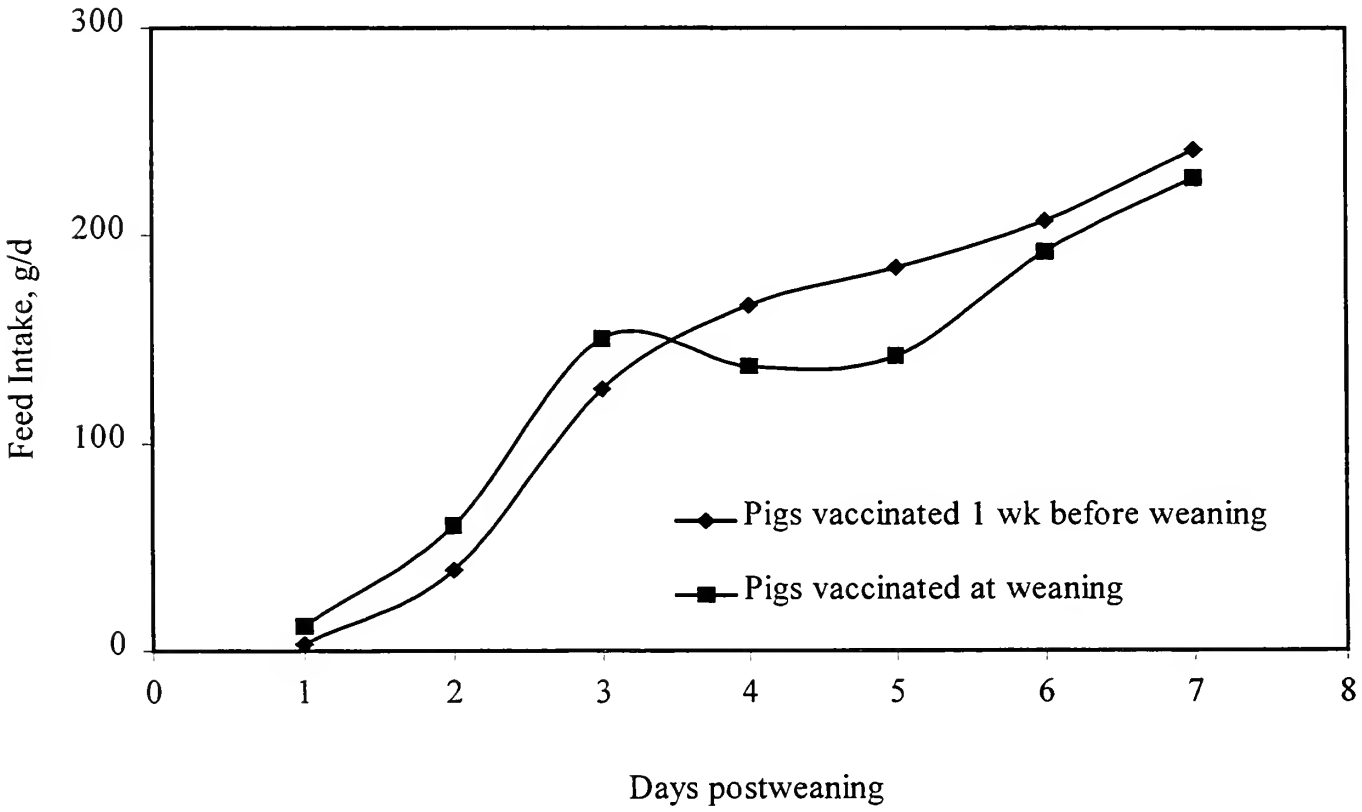

Figure 1. Daily feed intake pattern of pigs vaccinated 1 wk before weaning or at the time of weaning at 21 d-of-age.

Blend-Feeding Regimens for Nursery Pigs

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Up to five complex diets may be used in a phase-feeding program to accommodate the changing nutrient and ingredient requirements of fast-growing nursery pigs. Being expensive and prone to rapid quality deterioration, these diets require frequent mixing to ensure freshness and wholeness. Therefore, inputs such as logistics, storage capacity, and labor increase dramatically with a large number of diets.

Hand-feeding is frequently required for at least the first two postweaning diets because the quantity used is too small to be effectively handled by most automatic feeding systems. Hence, to reduce the number of manual feedings, a rather large quantity of diet is placed in feeders. But, when diets high in lactose and lysine (i.e., SEW, Phase 1 and 2 diets) are exposed to the warm and humid environment of a nursery facility, lysine bioavailability declines with time.

We are currently developing a different feeding program to reduce the number of diets required for maximal growth performance and to allow mechanization of frequent feedings. In a preliminary study, we compared a standard 3-phase feeding program with 3 diets against a regimen of several blends of only two diets. In the 3-phase program, Phase I diet was fed for 1 week postweaning, followed by Phase II for 2 weeks, and Phase III for another 2 weeks. In the blend-feeding program, Phase I and Phase III diets were blended in such proportions that pigs received more of the former and less of the latter diet after weaning and vice versa towards the end of the nursery period. The two blend programs differed in the amount and duration of feeding of each diet. In the aggressive regimen, Phase III diet replaced Phase I diet at a faster rate. All diets were fed in pellet form (5 mm).

Overall growth performance (Table 2) in terms of weight gain, feed intake, and feed efficiency, was not affected by any of the dietary treatments. In fact, under the moderate blend regimen pigs grew faster during the second week postweaning compared to the standard phase-feeding regimen. These results highlight the ability of blend regimens to support maximal performance and also the flexibility of this practice in matching the performance of pigs at the farm level, especially where closed-formula diets are bought from a commercial manufacturer.

In terms of cost of feed per pig, blends were slightly more expensive (i.e., $0.65 to $0.88 per pig). However, this was expected because allowances of Phase I diet were made deliberately high to allow expression of maximal performance without nutritional constrains. Currently, we are investigating other combinations of diets and different blending proportions to achieve maximal performance at a cost comparable or even lower than the feeding cost of standard nursery programs. Therefore, producers should not adopt this new practice until more information becomes available.

Table 1. Design of phase-feeding and blend-feeding nursery regimens

Week	Phase feeding regimen	Blend-feeding regimens			
		Moderate		Aggressive	
		Phase I	Phase III	Phase 1	Phase III
		--------- % ---------		--------- % ---------	
1	Phase I	100	0	100	0
2	Phase II	75	25	67	33
3	Phase II	50	50	33	67
4	Phase III	25	75	0	100
5	Phase III	0	100	0	100

Table 2. Performance and cost of phase-feeding vs blend-feeding nursery regimens

Item	Phase-feeding regimen	Blend regimen	
		Moderate	Aggressive
Weight gain, g/d			
Week 1	207	218	189
Week 2	201	311	267
Week 3	485	462	415
Week 4	475	547	516
Week 5	617	604	613
Overall	**397**	**428**	**400**
Feed intake, g/d			
Week 1	205	164	178
Week 2	354	392	360
Week 3	606	621	600
Week 4	690	754	823
Week 5	1029	1150	1069
Overall	**577**	**616**	**606**
Overall diet intake, kg			
Phase I	0.92	4.30	2.78
Phase II	4.32	-	-
Phase III	7.73	9.55	10.84
Cost/pig, $[a]	**12.97**	**13.85**	**13.62**

[a]Diet prices ($/ton): Phase I = 583; Phase II = 351; Phase III = 167.

Cost of Feeding Premixes In Late-Finishing Diets

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Vitamin and trace mineral premixes can be omitted from diets of late-finishing pigs (200 lb to market) without any negative effect on growth performance, carcass characteristics, meat quality, or animal health (Tables 1 and 2). Finishing pigs may not require any supplemental vitamins and trace minerals from premixes because overfeeding of vitamins and trace minerals during growing results in substantial body stores that can be drawn upon to cover any deficits. Moreover, coprophagy can be a substantial source of trace minerals and vitamins (microbial origin). Although maximal performance of healthy finishing pigs can be achieved without supplemental vitamins and trace minerals, omitting such premixes from the diets of diseased, growing, or breeding animals of any age is not recommended.

On the negative side, the concentration of vitamins and minerals in the meat from pigs fed diets without these premixes is expected to be reduced compared to the concentration in the meat of pigs fed fortified diets. The muscle depletion that might occur with some vitamins and trace minerals could be of nutritional importance to humans who rely on pork meat consumption to meet their micronutrient requirements. Nevertheless, the reduction of vitamin and trace mineral levels in pork meat, following omission of dietary premixes, has not been adequately characterized on a quantitative or qualitative basis. Moreover, there are no standards concerning optimal or maximal concentrations of any vitamin or trace mineral in pork meat so that producers, meat packers, and consumers could agree on the appropriate fortification levels of pig diets. Therefore, whether to omit premixes from late-finishing diets is a marketing decision, better left to the individual producer and its packer plant.

The economics of omitting vitamin and trace mineral premixes from late-finishing diets are presented in Table 3. It should be noted that these figures are subject to modification based on animal performance, and local premix inclusion rates and prices. In general, it is estimated that it costs over $4,000 per year to feed 10,000 late-finishing pigs (from 200 to 250 lb bodyweight) with vitamin and trace mineral premixes without any direct benefits to the producer.

The official position of the University of Illinois is to fortify all pig diets with vitamin and trace mineral premixes. Nevertheless, many universities and feed manufactures have already decreased the inclusion levels of their premixes for most finishing diets.

Table 1. Vitamins and trace minerals in late-finishing diets

Item	Corn	Soybean meal (47%)	Dicalicum phosphate	Limestone	Corn (83%) Soybean (14%) Dicalcium (0.8%) Limestone (0.7%)	Requirement (NRC, 1998) 200 to 250 lb
Vitamin A, µg/kg	327	-			-	447.2
Vitamin D, µg/kg[a]	-	-			-	3.75
Vitamin E, mg/kg	8.3	2.3			7.2	11.0
Vitamin K, mg/kg[b]	-	-			-	0.5
Niacin, mg/kg[c]	0	22.0			3.1	7.0
Pantothenic acid, mg/kg	6.0	15.0			7.1	7.0
Riboflavin, mg/kg	1.2	3.1			1.4	2.0
Vitamin B_6, mg/kg	5.0	3.2			4.4	1.0
Vitamin B_{12}, µg/kg[d]	-	-			-	5.0
Iron, mg/kg	29	176	7,900	3,500	136.40	40.00
Zinc, mg/kg[e]	18	55	-	-	22.64	50.00
Copper, mg/kg	3	20	-	-	5.29	3.00
Manganese, mg/kg	7	36	1,400	200	23.45	2.00
Iodine, mg/kg[f]	-	-	-	-	-	0.14
Selenium, mg/kg[g]	0.10	0.27	-	-	0.12	0.15

[a]Vitamin D is formed in the skin of animals with access to direct sunlight.
[b]Usualy satisfied by microbial synthesis.
[c]Corn contains about 24 mg/kg niacin but most of it is unavailable.
[d]It requires about 5 years to develop a B_{12} deficiency.
[e]Galvanized equipment are a source of zinc.
[f]Sea and iodized salts are rich sources of iodine. Body stores of iodine can last about 100 days.
[g]Certain areas of the Midwest produce grains with much lower selenium concentrations.

Table 2. Effects of omitting vitamin and(or) trace mineral premixes from late-finishing diets[a]

Item[b]	Control	Premix omitted		
		Vitamins	Trace mineral	Vitamins & trace minerals
ADG, g	1,097	1,047	1,103	1,050
ADFI, kg	3.54	3.23	3.33	3.30
Gain/feed, g/kg	311	325	330	318
Dressing percentage	74.1	74.4	74.3	74.5
10th rib fat thickness, mm	19.1	18.2	18.6	18.8
Longissimus muscle depth, cm	5.45	5.68	5.74	5.68
Longissimus muscle area, cm^2	48.9	51.3	49.2	48.9
Fat-free lean index, %	50.2	50.5	50.4	50.3
Water-holding capacity	2.16	2.38	2.36	2.32
Longissimus muscle color	2.6	2.5	2.5	2.5
Longissimus muscle firmness	2.5	2.5	2.5	2.6
Longissimus muscle marbling	1.9	1.9	1.8	1.9
Longissimus muscle lightness	54.3	54.3	54.7	54.2

[a]A total of 128 pigs (eight pigs/pen and four pens/treatment) with an avg initial BW of 85.7 kg and an avg final BW of 115.8 kg.

[b]There were no differences among dietary treatments.

Adapted from Mavromichalis et al. (1999, J. Anim. Sci. 77:2700-2708).

Table 3. Cost analysis of feeding vitamin and trace mineral premixes during late-finishing

Pigs numbers and expected performance

Pigs/year	= 10,000
Weight range	= 200 to 250 lb
Feed/gain	= 3.7 (200 to 250 lb body)
Feed intake/pig	= **185 lb**

Premixes (pmx) specifications

Vitamin pmx inclusion	= 2.5 lb/ton (0.125%)
Trace min pmx inclusion	= 2.0 lb/ton (0.1%)
Vitamin pmx intake	= **0.231 lb/pig** (for the last 50 lb of gain)
Trace pmx intake per pig	= **0.185 lb/pig** (for the last 50 lb of gain)

Cost of feeding premixes during late finishing

Cost of vitamin pmx	= $1.60 per lb
Cost of trace pmx	= $0.34 per lb
Cost of feeding vitamin pmx	= $0.370 per pig (0.231 lb/pig × $1.60/lb)
Cost of feeding trace pmx	= $0.063 per pig (0.185 lb/pig × $0.34/lb)
Total cost of feeding pmxs	***= $0.433 per pig or $4,330 per year for every 10,000 pigs***

Valine Uptake by Lactating Porcine Mammary Tissue

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Introduction

Milk is the exclusive nutrient source for the neonate. Enhanced management practices and availability of highly selected sows have allowed for increased litter sizes. The ability of the sow to produce adequate milk for large litters is limiting to neonate growth and total pig production (Boyd & Kensinger, 1998). Many studies have focused on improving nutrition for the high-producing lactating sow to maximize sow productivity.

Free amino acids in the blood are taken up by the lactating mammary gland. Once inside the mammary epithelial cell, the amino acids undergo various intracellular metabolic fates. The rate of protein synthesis is dependent upon three potential limiting steps: the amount of free amino acid available in the bloodstream, the ability of the transporter to take up the amino acid, and the various pathways the amino acid may enter once inside the cell.

Nutrition studies have shown that adding supplemental dietary lysine increases lactational performance of sows with large litters (Knabe et al. 1996). Supplemental lysine may affect the balance of plasma amino acids and alter mammary uptake of other amino acids, including valine (Knabe et al. 1996; Richert et al. 1996). Our previous studies have demonstrated that various amino acids, including branched chain amino acids (BCAA), can inhibit lysine uptake in sow mammary tissue (Schmid et al., 1999 Illinois Swine Research Report). Valine and lysine interactions can have physiological effects when supplemented in the sow diet. Nutrition studies also have found that supplemental dietary valine might increase litter weight gain (Richert et al. 1996; Tokach et al. 1993).

Valine and other BCAA are taken up by the mammary gland in quantities higher than their output in milk (Linzell et al. 1969, Trottier et al. 1997). Intracellular metabolic pathways other than protein synthesis may account for a significant portion of the BCAA taken up by the gland (Hurley & Bryson, 1997 Illinois Swine Research Report). Alternative fates of BCAA in the mammary gland, depending upon the amino acid, may include synthesis of cellular protein, synthesis of nonessential amino acids, utilization in fatty acid synthesis, and oxidization as an energy source. Animal nutrition studies do not usually account for these alternate metabolic pathways in the mammary gland.

Studies in the lactating mammary gland of other species have shown valine to be taken up via the system L transporter. Identified in mouse, rat, guinea pig, and cow, this transporter is generally specific for uptake of BCAA (Shennan 1998). However, the

system L transporter will also take up each of the neutral amino acids. The low specificity of the L transporter may mean that the balance of amino acids in the blood is particularly crucial to the transport of valine.

Valine uptake and intracellular metabolism is not well understood for the lactating sow at the mammary gland level. The objective of this study was to characterize ion dependency, kinetics, and specificity of the valine uptake system in the lactating sow mammary gland.

Experimental Approach

Lactating sows (day 15 of lactation or later) from the University of Illinois swine herd were slaughtered at the University of Illinois Meat Science Laboratory abattoir. Litters were removed immediately prior to slaughter. Mammary tissue was cut into fragments and incubated in the presence of media which demonstrated sodium dependency of valine uptake (culture media containing low vs physiological sodium concentrations), valine uptake kinetics (culture media containing valine concentrations ranging from 5 micromolar to over 5 millimolar), and competition by other amino acids (culture medium containing a low concentration of valine and supraphysiological concentrations of competitors).

Results

Uptake of valine increased with increasing medium concentration of valine. The kinetic analysis of valine uptake indicates that, within the range of physiological concentrations, the affinity of the valine uptake system is not limiting to valine uptake. Saturation of the valine transporter would not be expected to be a limiting factor when considering manipulation of blood valine levels.

System L has been characterized in the rat and cow as sodium independent (Shennan 1998; Baumrucker 1985). However in lactating sow mammary tissue, valine uptake was sodium dependent. This observation points out the variation in uptake mechanisms which exist among species.

Competition of valine uptake by high concentrations of leucine showed strong inhibition of valine uptake (Table 1). Even at physiological concentrations of leucine (320μM) valine uptake was inhibited by 47%. This is consistent with the specificity of the sow mammary tissue valine uptake system for BCAA.

Further competition experiments tested high concentrations of competitor amino acids. Several other amino acids showed inhibitory effects on valine (Table 1). Valine was inhibited by alanine (a short chain neutral amino acid), methionine (a sulfur containing short chain amino acid), glutamine (an amide derivative of an acidic amino acid), cyclo-leucine (a non-protein amino acid typically taken up by system L), and lysine (a cationic amino acid). The high concentrations of competitor amino acids used

in these experiments do not represent physiological competition, but the results do demonstrate that other amino acids besides the BCAA are potential competitors for valine uptake. Methyl aminoisobutyric acid (MeAIB) is a non-metabolizable substrate for system A, an uptake system that is principally responsible for the uptake of short-chain, neutral amino acids. Competition with MeAIB showed no significant inhibition even at supraphysiological concentrations, indicating that the valine transport system does have a level of specificity.

Conclusions

These studies have characterized fundamental kinetics and specificity of the valine transporter, and have added to our understanding of the nature and limitations of amino acid uptake mechanisms in porcine mammary tissue. An understanding of the valine uptake system is an important step in optimizing amino acid nutrition for lactating sows. Further studies are necessary to examine the intracellular metabolism of valine to understand its role in milk protein synthesis.

Acknowledgments

This project was supported in part by funding from the National Pork Producers Council and the Illinois C-FAR.

Table 1. *Inhibition of valine uptake by lactating sow mammary tissue by other amino acids.* [1]

Amino Acid Competitor	% Inhibition[2]
Leucine	90.67 ± 3.23
Alanine	88.83 ± 2.65
Lysine	67.22 ± 3.30
Cyclo-Leucine	80.72 ±3.08
Glutamine	85.31 ± 2.21
Methionine	90.75 ± 3.12
Methyl aminoisobutyric acid	18.28 ± 10.27

[1] Valine at 20 micromolar and competitors at 20.48 millimolar; n=6 sows

[2] Inhibition calculated as valine uptake in the presence of competitor as a percentage of uptake in the absence of competitor. Values are means ± standard error of the mean.

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Effect of Administration of PG600® to Sows at Weaning on the Time of Ovulation as Determined by Transrectal Ultrasound.

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Introduction

Reduced reproductive performance is often associated with season of the year (Love et al., 1993), lactation length, and sow parity (Hurtgen and Leman, 1981; Koketsu and Dial, 1997; Koketsu et al., 1997), and is observed frequently throughout the world. The reduction in reproduction originates from reduced numbers of sows returning to estrus, increased time to return to estrus, higher returns to estrus after mating, which collectively result in lower farrowing rates. Because suppressed gonadotropin secretion and ovarian inactivity are thought to be involved in some of these instances of lower reproduction, the use of exogenous gonadotropins has been investigated to stimulate ovarian activity in an attempt to alleviate this serious impediment to reproductive performance. Over the years, both PMSG (Britt, 1986) and PMSG/hCG (Lancaster et al., 1985; Bates et al., 1991; Kirkwood et al., 1998; Estienne and Hartsock, 1998) have been used on sows at weaning. Results of the exogenous gonadotropins on sow reproductive performance have generally been in agreement regarding the overall improvement in the percent of sows returning to estrus and the reduced time interval to return to estrus. However, some of the studies indicate an improvement (Lancaster et al., 1985; Britt, 1985) while others indicate no change or even slight declines in farrowing rates and litter sizes (Bates et al., 1991; Estienne and Hartsock, 1998; Kirkwood et al., 1998). The differences in the results of the studies are not unexpected when one considers the season of the study, differences in sow parity, and even differences in genetics, housing and management. However, despite these differences, most studies indicate that exogenous gonadotropins are effective at inducing sows into estrus when seasonal effects are most notable in non-treated sows, and are also effective at inducing more primiparous sows into estrus, which tend to have greater incidences of reproductive failure compared to multiparous sows. Taken as a whole then, the greater effective induction of estrus but generally little or no resulting improvement in farrowing rate is somewhat perplexing. Therefore this study was conducted in order to determine if the response of sows to PG600 at weaning was related to follicle development, ovulation, or altered time of ovulation relative to onset of estrus. This study also investigated how the time of breeding related to farrowing rate and litter size in sows induced into estrus with PG600.

Materials and Methods

The trial was conducted in 16 replicates coinciding with weaning over a one-year period of time. One hundred and thirty-seven sows of mixed parities were randomly assigned to receive

PG600® (n = 72) or no treatment (n = 65) at weaning (d 0). Sows received PG600® or no injection (control) on the day of weaning (d 0). Sows received PG600® in 5 cc of physiological saline intramuscularly in the neck using a 1½ inch 20 gauge needle. Sows were moved on day 0 to an estrous detection area where they were housed individually in gestation crates. On d 0, sows were moved to a breeding facility and observed for estrus twice daily at 0800 h and 1600 h using fence-line contact with a mature boar for 15 minutes. At onset of estrus and thereafter, transrectal ultrasound was performed twice daily at 0800 and 1600 h to determine average size of the three largest follicles and time of ovulation after onset of estrus. Ultrasound was performed using an Aloka 500V with a 7.5 MHz linear transducer attached to a PVC transrectal stabilizing rod. Sows were bred at 12 and 24 hours after first observed in standing estrus with 3.0 $x10^9$ sperm cells per insemination. The semen was not used more than 3-4 days after collection.

Housing and Management

Sows were kept in an environmentally regulated facility that maintained a constant air temperature and lighting regimen. Sows were fed once daily 2 kg of a 14% corn-soybean meal based diet.

Statistical Analysis

Data were analyzed using the GLM procedures of SAS for the continuous variables: time of ovulation from onset of estrus (hours), for follicle size at estrus (mm), and interval from d 0 to estrus, and litter size (total number of pigs born). The model for the continuous variables included (where appropriate): treatment, month, lactation length, interval from weaning to estrus, average of the three largest follicles at estrus, and their interactions. Categorical data involving the proportion of females expressing estrus, ovulating, and farrowing, were analyzed using the CATMOD procedure of SAS. The CATMOD model included both treatment and replicate. Farrowing rate and litter size were also analyzed using the GLM procedures of SAS, which included the independent variables; time to first service after onset of estrus (0, 12, 24, or 36 h) and the interval from time of the last insemination to ovulation (-36, -24,-12, 0, +12, +24 h).

Results and Discussion

The weaning age ranged from 10 to 34 d but the average weaning age (21.0 ± 0.5 d) was not different ($P > .10$) between treatment groups. More sows treated with PG600® at weaning expressed estrus within 10 days ($P < .01$) compared to controls (94% vs 78%). There was a tendency ($P = .09$) for more sows treated with PG600® to ovulate compared to control sows (90.3 vs 81.5%). The interval from weaning to estrus was reduced ($P < .001$) by PG600® compared to controls (3.8 vs 5.0 days). Follicle size at estrus was not affected by treatment and averaged 7.80 ± .01 mm. The interval from onset of estrus to ovulation was longer for sows treated with PG600® ($P = .05$) compared to controls (48 vs 41 hours). The effect of both PG600 on ovulation time and the interval from onset of estrus appeared to be important since PG600 sows expressed estrus earlier and ovulated later after onset of estrus. Overall, the time of ovulation after onset of estrus was negatively related ($r = -0.50$) with the interval from weaning to estrus ($P = .005$). The time of ovulation after onset of estrus was greatest ($P < .05$) for sows expressing estrus within 2 to 3 d of weaning compared to sows expressing estrus after 5 days and was shortest ($P < .01$) for sows expressing estrus by 6 d after weaning. The farrowing rate of sows was not significantly affected by treatment and averaged 70%. However, the farrowing rate

was influenced by time of last insemination relative to ovulation (P<.01), and more sows farrowed (>80%) when the last insemination occurred at –12 to 0 h before the time of ovulation. Total number of pigs born tended to be reduced for sows treated with PG600 (P =.10). Although not significant (P > .10), there appeared to an important relationship between the timing of the last insemination relative to time of ovulation and litter size. Litter sizes were ≥11.0 pigs per sow when last insemination occurred –24 h to +12 h of the time of ovulation.

Conclusion

This study indicates that PG600® can provide significant advantages for inducing estrus and ovulation across different months of the year. However time of breeding may need to be adjusted when using PG600® at weaning to optimized reproductive improvement when accounting for time of ovulation based on the interval from weaning to estrus.

Acknowledgements
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Table 1.

Effect of Administration of PG600® to Sows at Weaning on Measures of Reproductive Performance

TRT [a]	n [b]	dwean [c]	% Estrus [d]	destrus [e]	Follicle Size (mm) [f]	% Ovulate [g]	% of Estrus/Ovulate [h]
C	65	21.9 ± 0.5	78% (51/65)	5.0 ± 0.1	7.96 ± 0.02	81.5% (53/65)	96% (51/53)
PG600	72	21.1 ± 0.4	94% (68/72)	3.8 ± 0.1	7.72 ± 0.02	90.3% (65/72)	100% (68/65)
P Value[l]		NS	P<.01	P<.001	NS	P=.09	NS

[a]sows received PG600 at weaning or no treatment (C, control)
[b]number of sows randomly assigned to treatment
[c]days from farrowing to weaning (mean ± SEM)
[d]percent of treated sows expressing estrus within 10 days
[e]days from weaning to estrus
[f]mean of three largest follicles at estrus
[g]percent of treated sows ovulating
[h]percent of sows expressing estrus that ovulated
[l]not significantly different (NS), means within a column are different at P<.05.

Table 2.

Effect of PG600 Administered to Sows at Weaning on Time of Ovulation after onset of Estrus (Ovhr), Farrowing Rate (FR) and Total Number of Pigs Born (tNB).

TRT	Ovhr	FR (%)	tNB
C	41.4 ± 1.9 h	72%	11.9± 0.6
PG600	47.6 ± 2.3 h	68%	10.6± 0.5
P value[1]	P=.05	NS	P=.10

[1]not significantly different (NS), means are significantly different within a column at P<.05.

The Effects of the Phytoestrogen, Daidzein on *In Vitro* Development of Porcine Embryos

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Project Summary
One of the most critical aspects of swine reproduction is embryonic development. Embryonic development is directly related to litter size which also indicates the reproductive performance of a sow or gilt. In pig reproduction, it is know that estrogen plays a major role in early embryonic development and maternal recognition of pregnancy. Maternal recognition of pregnancy is a reproductive process in which little is known. Nonetheless, it is known that a minimum of four embryos must be present in the uterus 12-15 days after fertilization to maintain pregnancy. One of most important factors with maternal recognition of pregnancy in the pig is the production of estrogen by the embryo. This estrogen production by the embryo prevents PGF_2 alpha from being secreted into the lumen of the uterus. The prevention of PGF_2 alpha secretion into the lumen of the uterus allows the embryos to continue to develop. However, the mechanism for estrogen production by the embryo is unknown. It is not known whether the embryo produces the estrogen or converts another substance into estrogen.

There are many estrogenic isoflavones called phytoestrogens found in the products of soybeans. Presently, the role of the isoflavones in early embryonic development in the pig has not been investigated. Isoflavones may play a role in embryonic development and also gene expression of the early embryos. Gene expression of the early embryo may be important by allow certain growth factors to be turned on or off to increase embryo survival. An increase in the number of embryos surviving directly increases the litter size and improves reproductive performances. With Illinois being one of the top five hog and soybean producing states in the U.S., it is appropriate to conduct the research to determine the role of isoflavones in porcine embryonic development and embryonic gene expression. This research may help increase the consumption of soybean based products in swine diets.

Project Objective
To determine the effect of daidzein on development *in vitro* of porcine embryos.

Introduction
Early embryogenesis and factors that affect early embryogenesis is an important area of research at the University of Illinois. Proper embryonic development is important for embryo survival, which directly correlates to litter size and reproductive performance.

Growth factors are critical in the control of animal cell growth (Simmen et al., 1990). The regulation and exposure of growth factors is becoming an important mechanism that needs to be investigated. It is known that growth factors work with the second messenger system, such as cyclic adenosine monophosphate (cAMP), phosphoinositol or calcium effector pathway (Hill,

1991). There are intercellular mechanisms involved with growth and maturation of tissues classically controlled by hormones such as estrogen (Simmen et al., 1988).

There has been some investigation into the effects of growth factors on embryo development in the mouse and bovine. The growth factors that have been investigated are insulin-like growth factor II, platelet-derived growth factor, epidermal growth factor, uterine-derived growth factor, and colony-stimulating factor-1. However, there has been some investigation into the effects of growth factors in swine. Abeydeera et al., (1997) reported that epidermal growth factor had no beneficial effect on swine blastocyst development *in vitro*. However, epidermal growth factor was shown to have a beneficial effect on subsequent development of *in vitro* maturated and fertilized pig embryos when added to the maturation medium. Uterine luminal fluid mitogen (ULFM) is a growth factor that promotes DNA synthesis in cultures of porcine uterine cells in domestic pigs and might regulate uterine growth and/or differentiation (Simmen et al., 1988). On day 12 of gestation, insulin-like growth factor I (IGF-I) is at it's highest level and appears to be regulated by estrogen. In the pig embryo, it is not know whether the embryos produces estrogen, acquires the estrogen from the uterine lumen fluid or converts another substance to estrogen. It has been shown that IGF-I promotes protein synthesis in porcine embryonic discs *in vitro* (Estrada et al., 1991).

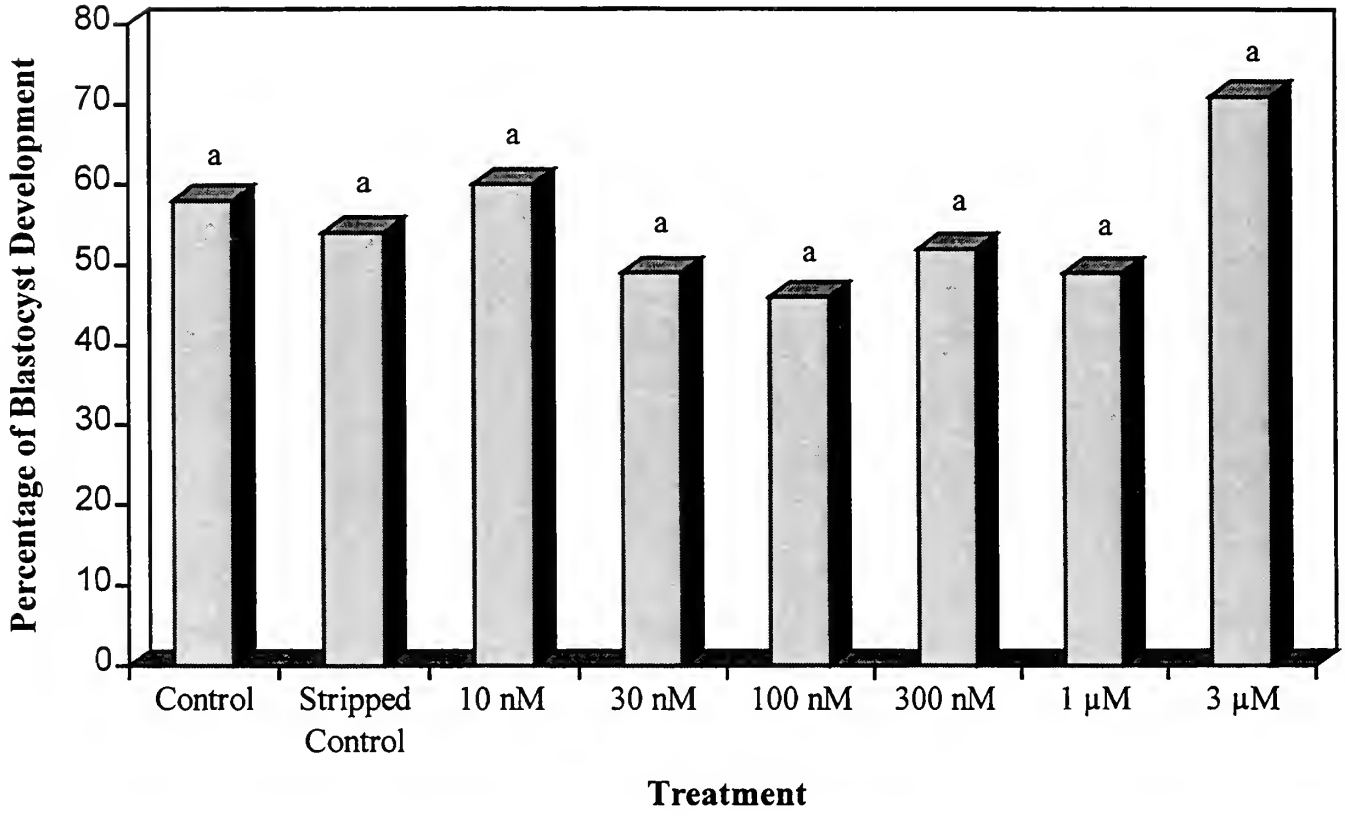
Materials and Methods

Prepubertal gilts were superovulated with an injected PG600® at approximately 200 days of age followed by an injection of human chrionic gonadotropin (hCG) 72 h later. Gilts were mated to produce hybrid embryos. Two-cell embryos were collected surgically from the donor gilts. Following collection, two cell embryos were cultured with and without increasing doses (0, 10nM, 30nM, 100nM, 300nM, 1μM, 3μM) of daidzein to determine the effect on developmental rates. The base medium was North Carolina State University 23 with a 0.4% charcoal stripped bovine serum albuim. The embryos were cultured for 96 hours and stage of development was evaluated and recorded every 24 h. However, a portion of the embryos reaching the blastocyst stage were saved for RT-PCR. The remaining blastocyst were fixed and stained with propidium iodine and Hoechst 33342 to determine the inner cell mass number as well as the total cell number. Development data was analyzed by chi-squared analysis with the aid of the computer-based program Statview 4.0 (Abacus Concepts, Inc).

Conclusions

The phytoestrogen daidzein has been reported to be a reagent that arrest cells in the G1 portion of the cell cycle (Higashi and Ogawara, 1994). It has been reported that estrogen is required for the transition from the compacted morula stage to the blastocyst (Niemann and Elsaesser, 1986). The results from this study were that there was no significant difference ($p<0.05$) in the formation of blastocyst between embryo cultured with 1 μM (20/41 49%), 10 nM (24/40 60%), 100 nM (18/39 46%) compared to the control embryos (25/43 58%). Embryos cultured in the presence of 30 nM (21/43 49%), 300 nM (22/42 50%) also had no significant difference ($p<0.05$) compared to controls. However, embryos cultured in the presence in 3 μM (30/42 71%) exhibited an increase in the percentage of blastocyst formation although it was not statistically significant ($p<0.05$) as seen in Figure 1. The 3 μM embryo data may help support the theory that estrogen is required for the transition from the compacted morula to blastocyst. Cell counts may also be helpful in determining the quality of the 3 μM treated blastocyst compared to control.

FIGURE 1. PERCENTAGE OF BLASTOCYST DEVELOPMENT OF PIG EMBRYOS CULTURED IN THE PRESENCE OF DAIDZIEN



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Myostatin (GDF-8) as a potential quantitative trait locus in swine

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Introduction

Myostatin (GDF-8) is a member of the TGF-β superfamily of growth factors which is expressed predominantly in skeletal muscle. Myostatin is a purported negative regulator of muscle development (McPherron et al., 1997). Myostatin knock-out mice (mice which have the myostatin gene specifically inactivated) have individual muscles which can weigh 2 to 3 times more than the same muscles in wild-type control mice. Furthermore, the double-muscled body type of Belgian Blue and Piedmontese cattle has been linked to an inactive myostatin gene (McPherron and Lee, 1997). The loss of myostatin activity causes these cattle to be extremely muscular and lean. Porcine studies have shown that runt piglets have increased myostatin expression when compared to the more heavily muscled control piglets (Shaoquan et al., 1998).

The production of animals with superior muscle structure is of great importance to food animal agriculture. Skeletal muscle is the major component of lean tissue that is used for food. In swine, there are observable differences in the carcass muscularity of different breeds of pigs. For example, Yorkshire pigs have shown a greater ability to synthesize proteins within muscle (Ezewke and Martin, 1975) as well as having greater muscle weights than feral pigs of equal weight or age (Buhlinger et al., 1978). Chinese Meishan pigs, on the other hand, have been shown to have poor growth rates and efficiency and fatty carcasses when compared to the occidental breeds (Gianola et al., 1982). Due to its role in muscle development in other mammals, it is important to investigate myostatin's role in swine muscle development. In swine, sequence variations in the GDF-8 coding region may lead to varying levels of myostatin expression among breeds and subsequently determine variation in muscle development. These same sequence differences may potentially serve as markers for muscle traits in swine. Producers often use genetic markers for herd and production management. GDF-8 may represent an ideal marker for muscle mass because it is a single gene, the absence of which has shown to increase muscle mass in both mice and cattle.

The objectives of this study were: 1) To compare the myostatin coding region sequence between Meishan, Yorkshire, Duroc and Hampshire pigs and 2) To establish a developmental comparison of myostatin mRNA expression between Meishan and Yorkshire piglets.

Materials and Methods

GDF-8 coding region sequence analysis. This experiment was designed to examine potential variations in the porcine GDF-8 coding region sequence between Duroc, Hampshire, Meishan and Yorkshire breeds of pigs. The Chinese Meishan pig is not a production animal in the U.S., but because of it's decreased level of muscle development it is an excellent resource for this study (White et al., 1995). Total RNA was isolated from the gluteus muscle of market weight hogs for each of the four breeds. GDF-8 specific primers were developed to amplify the coding region in an reverse-transcription polymerase chain reaction reaction (RT-PCR). The RT-PCR products were cloned into a TA cloning vector (Invitrogen, Carlsbad, CA), propagated and

appropriate colonies selected for sequencing. Sequenced samples were compared to the already published sequence (McPherron and Lee, 1997) and to each other. We analyzed the sequences for additions, deletions and/or single-base variations.

Myostatin mRNA expression determination. To supplement the GDF-8 sequence analysis, we have also tried to establish a developmental comparison of myostatin expression between Meishan (low muscle/high fat) and Yorkshire (higher muscularity) piglets. Animals for this study were selected at day 54 of gestation as well as day 14, 30 and 60 post parturition. Total mRNA was isolated from rump muscle of Meishan and Yorkshire piglets at the previously mentioned ages. RT-PCR was used to design a 630-bp radiolabeled probe that could be used to hybridize to the target GDF-8 sequence in Northern analysis. The produced autoradiograph allowed us to quantify the myostatin expression through the use of the Collage™ image analysis software. We analyzed the different age and breed samples (24 total) on one single blot. Using one blot allowed us to compare GDF-8 expression between Meishan and Yorkshire breeds at corresponding days of development as well as expression in the same breed at the four different ages of development.

Results and Discussion

GDF-8 coding region sequence. The 1128 base pair GDF-8 coding region was sequenced for one Duroc, one Hampshire, two Meishans and one Yorkshire pig and compared to an already published sequence as well as to each other. Variations in this region of the gene may potentially modify GDF-8 expression by altering the amino acid structure of myostatin. Upon analyzing the sequences, it was determined that the GDF-8 coding region sequence (Figure 1) was identical in all four breeds. We were unable to identify any additions, deletions or point variations. It was hypothesized that, given the role of myostatin in muscle development, the Meishan GDF-8 coding region would show some sequence variation when compared to the more heavily muscled domestic breeds.

Myostatin mRNA expression. Myostatin's role as a negative regulator of muscle development has been well established in mice and cattle. Decreased or the absence of myostatin expression in both cattle and mice has been shown to result in dramatic increases in lean muscle mass. We were interested to determine if the same direct relationship was found in pigs. To do this, we examined myostatin expression in two breeds with extremely different muscularities: Meishan and Yorkshire pigs. The study was designed to examine how myostatin expression changes as piglets develop and also how Meishan myostatin levels compare to those of same age Yorkshire piglets. The data obtained illustrates a trend of increased myostatin expression in Yorkshire piglets as compared to the Meishan piglets for all four time points sampled (Figure 2). Increased levels of myostatin expression in the Yorkshire samples at days 14, 30 and 60 as compared to the respective Meishan samples was significant ($P<.05$). The data also indicates a trend of increased myostatin expression in the Meishan breed as progression from day 54 pre-partum to day 60 post-partum (Figure 3). The only significant difference ($P<.0134$) in expression was found when the day 54 pre-partum were compared to the day 60 samples. The Yorkshire samples illustrated a similar trend of increased myostatin expression as the age of development increases (Figure 3). Significant differences were found in the following Yorkshire comparisons: day 54 pre-partum and day 30 ($P<.0042$), day 54 pre-partum and day 60 ($P<.0056$), day 14 and day 30 ($P<.0131$), and day 14 and day 60 ($P<.0158$). These data indicate that myostatin expression increases as both Meishan and Yorkshire piglets develop and that Yorkshire piglets express GDF-8 mRNA at higher levels than Meishan piglets of the same age. Because of GDF-8's role as a negative

regulator of muscle development, we hypothesized that the less muscular Meishan pigs may have higher levels of myostatin expression than the muscular Yorkshire pigs. These results indicate that GDF-8 expression in swine may not have such a direct correlation to decreased muscle development.

Conclusions

1. The GDF-8 coding region sequence was identical in Duroc, Hampshire, Meishan and Yorkshire pigs.

2. Yorkshire pigs exhibit a higher level of myostatin mRNA expression than the Meishan pigs of the same ages.

3. The data from this study indicate that, at this point, myostatin does not make a useful quantitative trait locus in swine and that the role of porcine myostatin as a negative regulator of muscle development is not as straight forward as it seems to be in the bovine system.

Acknowledgements

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1    atgcaaaaac tgcaaatcta tgtttatatt tacctgttta tgctgattgt tgctggtccc
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121  gcatgtatgt ggagacaaaa cactaaatct tcaagactag aagccataaa aattcaaatc
181  ctcagtaaac ttcgcctgga aacagctcct aacattagca aagatgctat aagacaactt
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1081 tatgggaaaa ttccagccat ggtagtagat cgctgtgggt gctcatga
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Figure 1. Porcine myostatin mRNA sequence which was found to be identical in the Duroc, Hampshire, Meishan and Yorkshire pigs sampled.

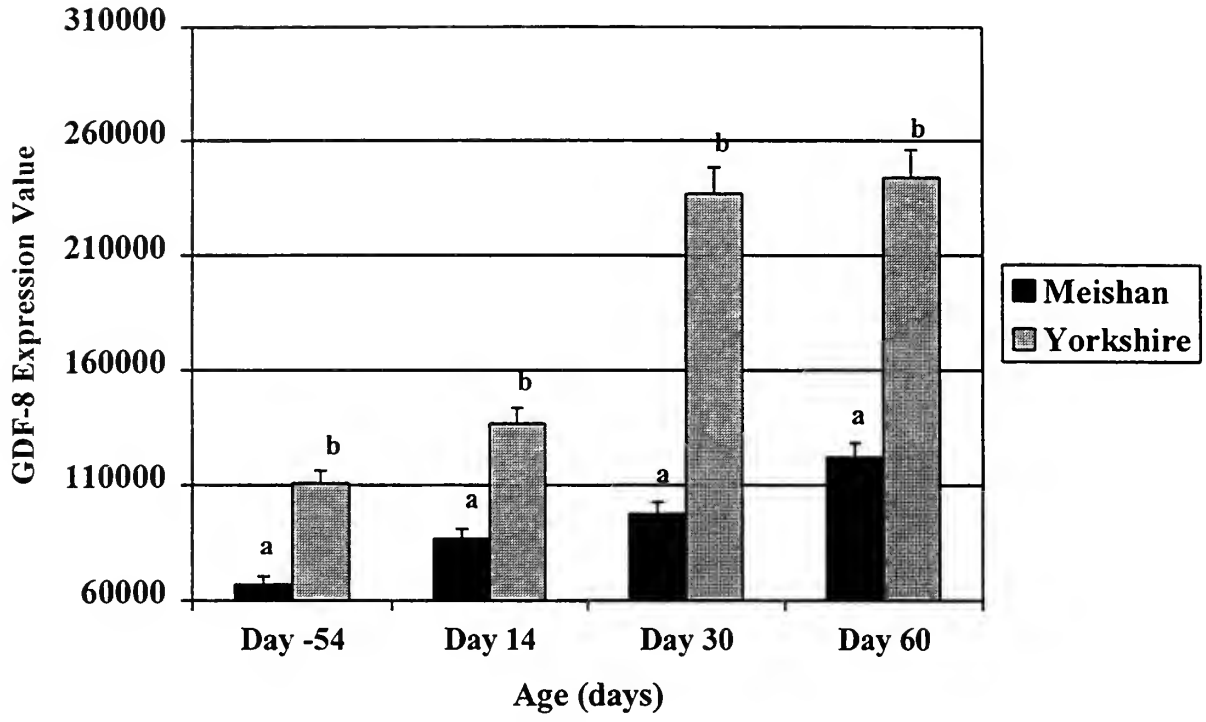

Figure 2. Comparison of GDF-8 expression between Meishan and Yorkshire piglets at day 54 of gestation as well as day 14, 30 and 60 post parturition. Values with different superscripts are significantly different at P<.05.

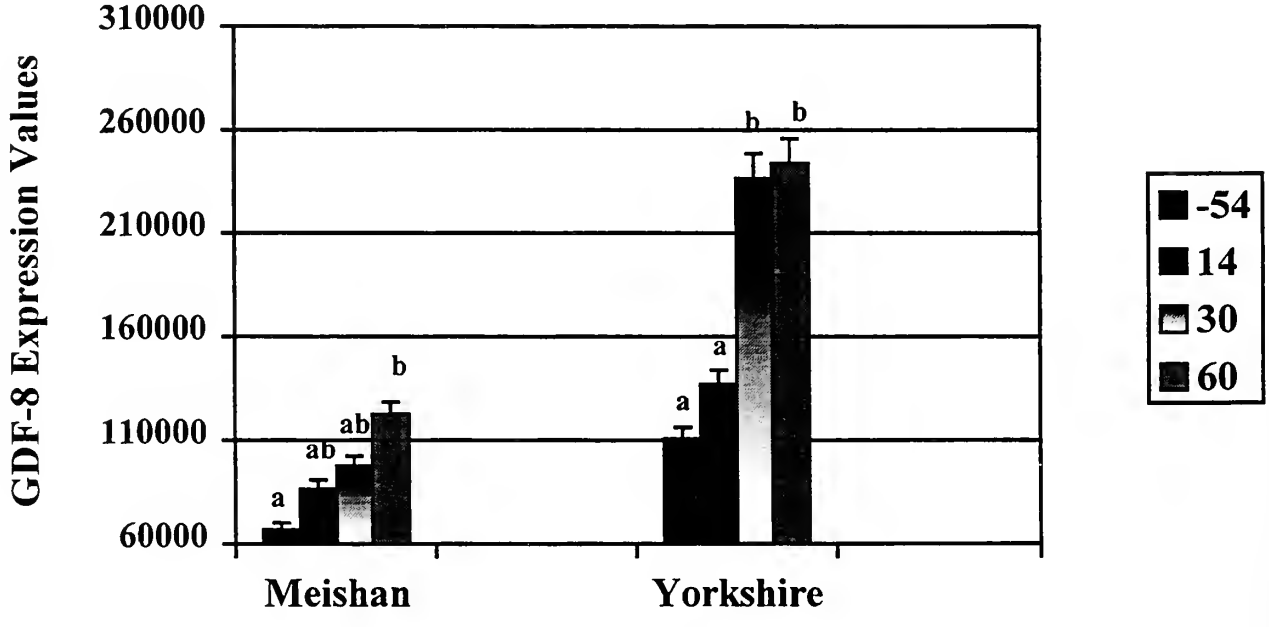

Figure 3. Comparison of GDF-8 expression in Meishan and Yorkshire pigs at day 54 of gestation as well as day 14, 30 and day 60 post parturition. Within breeds, values with common superscripts do not differ. Values with different superscripts are different at P<.05.

Growth-Promoting Efficacy of Tetrabasic Zinc Chloride and Two Sources of Zinc Oxide for Newly Weaned Pigs

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Pharmacologic levels of supplemental Zn are well established as growth promoters for newly weaned pigs. Feed-grade zinc oxide (ZnO) manufactured by the Waelz process dominates the feed-grade Zn market for pigs, and this source of ZnO also has been most heavily researched as a growth promotant for nursery pigs (Hahn and Baker, 1993; Hill *et al.*, 1999; Mahan *et al.*, 2000; Mavromichalis *et al.*, 2000). Our research with chicks has demonstrated that Waelz ZnO (72% Zn) has a relative Zn bioavailability (RBV) of around 43% relative to analytical-grade ZnO (80.3% Zn). Another source of feed-grade ZnO is manufactured by the hydrosulfide process and contains about 78% Zn. Our research has shown that the RBV of Zn in this product is somewhat higher than that in analytical-grade ZnO (Edwards and Baker, 1999).

A new source of Zn, tetrabasic zinc chloride (TBZC) with the formula $Zn_5Cl_2(OH)_8$ (containing about 60% Zn) is now available to the feed industry. The RBV of Zn in this product is about 20% higher than that in analytical-grade ZnO (Corless *et al.*, 2000). Unlike Waelz ZnO which contains substantial quantities of cations other than Zn (e.g., 1.8% Fe, 1.7% Ca, 0.38% Na, 0.19% Mn, 0.16% Mg, and 0.10% Al), TBZC contains only traces of these contaminating cations.

The growth-promoting ability of ZnO (or other sources of Zn) has taken on added significance in recent years because of the resistance-cross resistance concerns inherent in using antibacterial/antibiotic supplements in pig rations. Thus, routine antibiotic use in animal feeds (for growth promotion) may become illegal in the years ahead. The antibacterial properties of ZnO are well established in human medicine (Sordeberg *et al.*, 1990), and therefore researchers focusing on early nutrition of nursery pigs are greatly interested in the growth-promoting efficacy of pharmacologic levels of Zn for nursery pigs.

Illinois and United Feeds, Inc., Experiments. A typical phase-I nursery diet (100 mg Zn/kg provided in trace-mineral premix) was used in all experiments, and pigs were of either PIC or BMI genetics. The pigs were housed in nursery pens with woven-wire floors in environmentally-controlled nursery buildings. The first three experiments (Tables 1-3) compared a supplemental ZnO source with a low Zn RBV (Waelz process) to a ZnO source having a high Zn RBV (Hydrosulfide process). The supplemental Zn level was 1500 mg/kg in all of these experiments. Experiments 4 and 5 (Tables 4 and 5) were designed to compare Waelz ZnO to $Zn_5Cl_2(OH)_8$ (TBZC) at varying supplemental Zn levels, either in diets without (Exp. 4) or with added antibiotics i.e., ASP-250 (Exp. 5).

Experiment 1 (Table 1). Pigs in this experiment were given a 7-d postweaning adjustment period during which neither supplemental Zn nor antibiotics were fed. During the subsequent 21-d assay period, 1,500 mg Zn/kg was fed from either Waelz (W) ZnO or hydrosulfide (HS) ZnO, and no antibiotics were present in any of the assay diets. The lower dose (1,500 mg Zn/kg) of ZnO from a low RBV source (W) or a high RBV source (HS) was selected because it was felt that a less-than-optimal dose of ZnO would better detect potential efficacy differences between the two sources of ZnO.

During the 1st week of the assay, both sources of ZnO elicited a marked gain and feed efficiency response ($P < 0.01$), and the responses were greater ($P < 0.07$) for HS than for W ZnO. For the entire 21-d feeding period, however, efficacy differences between W and HS ZnO did not exist. Also, in this trial, the weight gain response elicited by supplemental ZnO was due primarily to increased feed intake. Gut morphology (data not shown) was examined at trial termination in five pigs (medium-weight pig in each pen) per treatment, but ZnO supplementation had little effect on villus height or width or on crypt depth.

Experiment 2 (Table 2). No antibiotics were present in the diets fed in this trial, but unlike the 7-d postweaning pretest feeding period of Exp. 1, the pigs in Exp. 2 were started on trial immediately after weaning at 21 d. A growth response ($P < 0.05$) averaging 26% and a feed efficiency response ($P < 0.01$) averaging 16% occurred due to 1500 mg Zn/kg added ZnO, regardless of ZnO source.

Experiment 3 (Table 3). A 7-d postweaning pretest feeding period was employed in Exp. 3, and an antibiotic premix (ASP-250) was present in the diet during both the 7-d pretest period and the 11-d ZnO test period. Supplemental ZnO, regardless of source, increased both weight gain ($P < 0.06$) and feed efficiency ($P < 0.05$) during the initial 6 d and the entire 11 d of the experimental feeding period. There appeared to be some advantage in feed efficiency for the ZnO source (HS) that was high in Zn RBV over the source (W) that was low in Zn RBV.

Experiment 4 (Table 4). Pigs were weaned at 15 d of age and placed immediately on experimental diets containing variable levels of Waelz ZnO or TBZC, but with no antibiotics. Feeding graded levels of supplemental Zn from ZnO produced a linear ($P < 0.06$) response in both gain and gain/feed ratio. It appeared that the 3,000 mg Zn/kg dose from ZnO was required in this trail to stimulate weight gain. The quadratic ($P < 0.07$) gain response to TBZC suggested that 1,500 mg Zn/kg from TBZC was as effective as 3,000 mg Zn/kg for enhancing weight gain, although the feed efficiency response to TBZC was linear when Zn doses of 0, 1,500 and 3,000 mg/kg from TBZC were evaluated statistically. Averaged over both doses of supplemental Zn, TBZC produced a greater feed efficiency response than ZnO.

Experiment 5 (Table 5). With ASP-250 present in all diets, early weaned pigs did not show a weight gain response to 1,500 mg Zn/kg addition in this experiment. Feed efficiency assessment, however, indicated a highly significant ($P < 0.01$) response to TBZC but not to Waelz ZnO.

Conclusions: General conclusions from the experiments presented, plus others not shown here are itemized below.

1. Pharmacologic Zn dosing of early weaned or conventionally weaned pigs provides consistent and meaningful responses in pig performance.

2. Pharmacologic levels of Zn are more reliable than pharmacologic levels of Cu (i.e., copper sulfate) for growth promotion in nursery pigs.

3. ZnO or TBZC are efficacious Zn sources for nursery pigs in both the absence and presence of feed additive levels of antibiotics.

4. Zinc sulfate does not give consistent growth responses when fed to provide pharmacologic levels of dietary Zn.

5. At lower doses (i.e., 1,500 mg Zn/kg), there are performance advantages for a ZnO source whose Zn bioavailability is high (hydrosulfide-processed ZnO) compared with a ZnO source whose Zn bioavailability is low (Waelz-processed ZnO).

6. In the absence of dietary antibiotics, 3,000 mg Zn/kg from Waelz ZnO produces greater efficacy than 1,500 mg Zn/kg.

7. Tetrabasic Zn chloride (TBZC) is an excellent growth promoter, and 1,500 mg Zn/kg from TBZC is as effective as higher levels for stimulating weight gain and feed efficiency of newly weaned pigs consuming phase I diets in either the absence or presence of antibiotics.

8. Although pharmacologic Zn dosing with ZnO or TBZC consistently improves performance of nursery pigs, weight gain sometimes responds more than feed efficiency, but feed efficiency sometimes responds more than weight gain.

9. When evaluated immediately postweaning, responses to pharmacologic Zn levels often are not observed until after the first week of supplemental Zn feeding.

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Table 1. Effects of ZnO source on growth performance of nursery pigs fed diets without antimicrobial agents (Exp. 1)[a, b]

	Dietary treatment[c]				Contrasts, *P*-value[c, d]	
Item	No added ZnO	1,500 mg Zn/kg (Waelz)	1,500 mg Zn/kg (Hydrosulfide)	SEM	None vs ZnO	W vs HS
Day 0 to 7						
Weight gain, g/d	145	242	304	17	0.01	0.05
Feed intake, g/d	279	375	414	17	0.01	NS
Gain/feed, g/kg	520	645	734	34	0.01	0.07
Day 7 to 14						
Weight gain, g/d	375	443	501	21	0.01	0.08
Feed intake, g/d	473	569	604	27	0.01	NS
Gain/feed, g/kg	793	779	829	34	NS	NS
Day 14 to 21						
Weight gain, g/d	375	425	395	29	NS	NS
Feed intake, g/d	620	657	701	26	NS	NS
Gain/feed, g/kg	604	648	563	37	NS	NS
Day 0 to 21						
Weight gain, g/d	298	370	400	15	0.01	NS
Feed intake, g/d	457	534	573	17	0.01	NS
Gain/feed, g/kg	652	693	698	24	NS	NS

[a]Data are means of five pens of four pigs with an average initial body weight of 6.2 kg during a 21-d growth assay.

[b]During a 7-d postweaning pretest period (21 to 28 d of age), all pigs had access to the basal diet containing no added ZnO or antimicrobial agents.

[c]W = Waelz-processed ZnO (low Zn bioavailability); HS = hydrosulfide-processed ZnO (high Zn bioavailability).

[d]NS = not significant $P > 0.10$.

Table 2. Effects of ZnO source on growth performance of postweaned pigs fed diets without antimicrobial agents (Exp. 2)[a]

	Dietary treatment[b]				Contrasts, *P*-value[b, c]	
Item	No added ZnO	1,500 mg Zn/kg (Waelz)	1,500 mg Zn/kg (Hydrosulfide)	SEM	None vs ZnO	W vs HS
Weight gain, g/d	218	276	271	19	0.05	NS
Feed intake, g/d	306	340	321	26	NS	NS
Gain/feed, g/kg	712	812	844	24	0.01	NS

[a]Data are means of four pens of four pigs with an average initial body weight of 5.4 kg and an average initial age of 21 ± 2 d during a 17-d feeding period.
[b]W = Waelz-processed ZnO (low Zn bioavailability); HS = hydrosulfide-processed ZnO (high Zn bioavailability).
[c]NS = not significant $P > 0.10$.

Table 3. Effects of ZnO source on growth performance of nursery pigs fed diets containing antimicrobial agents (Exp. 3)[a, b]

	Dietary treatment[c]				Contrasts, *P*-value[c, d]	
Item	No added ZnO	1,500 mg Zn/kg (Waelz)	1,500 mg Zn/kg (Hydrosulfide)	SEM	None vs ZnO	W vs HS
Day 0 to 6						
Weight gain, g/d	144	187	216	22	0.06	0.10
Feed intake, g/d	289	323	325	22	NS	NS
Gain/feed, g/kg	498	579	665	39	0.05	NS
Day 0 to 11						
Weight gain, g/d	208	250	265	21	0.06	NS
Feed intake, g/d	428	422	403	37	NS	NS
Gain/feed, g/kg	486	592	658	24	0.01	0.09

[a]Data are means of five pens of four pigs with an average initial body weight of 6.2 kg during an 11-d growth period.
[b]During a 7-d postweaning pretest period (21 to 28 d of age), all pigs had access to the basal diet containing no added ZnO but containing antimicrobial agents.
[c]W = Waelz-processed ZnO (low Zn bioavailability); HS = hydrosulfide-processed ZnO (high Zn bioavailability).
[d]NS = not significant $P > 0.10$.

Table 4. Effects of increasing doses of zinc from zinc oxide or tetrabasic zinc chloride on growth performance of postweaned pigs fed diets without antimicrobial agents (Exp. 4)[a]

Treatments[b]	Weight gain, g/d	Feed intake, g/d	Gain/feed, g/kg
1. No added Zn (control)	234	329	711
2. 1,500 mg Zn/kg from ZnO	240	341	705
3. 3,000 mg Zn/kg from ZnO	265	363	730
4. 1,500 mg Zn/kg from TBZC	263	353	745
5. 3,000 mg Zn/kg from TBZC	261	339	769
Pooled SEM	11	13	14
Contrasts, P-value[c]	0.05	NS	0.01
Control vs added Zn	NS	NS	0.01
ZnO vs TBZC	0.06	NS	NS
ZnO linear	NS	NS	NS
ZnO quadratic	0.06	NS	0.01
TBZC linear	0.07	NS	NS
TBZC quadratic			

[a]Data are means of five pens of six pigs with an average initial body weight of 5.2 kg and an average initial age of 15 ± 2 d during a 21-d feeding period.
[b]ZnO (Waelz) vs. tetrabasic Zn chloride (TBZC).
[c]NS = not significant $P > 0.10$.

Table 5. Effects of 1,500 ppm of zinc from zinc oxide or tetrabasic zinc chloride on growth performance of postweaned pigs fed diets with an antimicrobial agent (Exp. 5)[a]

Treatments[b]	Weight gain, g/d	Feed intake, g/d	Gain/feed, g/kg
1. No added Zn (control)	282	388	725
2. 1,500 mg Zn/kg from ZnO	280	392	718
3. 1,500 mg Zn/kg from TBZC	295	383	769
Pooled SEM	10	13	11
Contrasts, P-value[c]			
Control vs ZnO	NS	NS	NS
ZnO vs TBZC	NS	NS	0.01

[a]Data are means of eight pens of six pigs with an average initial body weight of 5.2 kg and an average initial age of 16 ± 2 during a 19-d feeding period.
[b]ZnO (Waelz) vs. tetrabasic Zn chloride (TBZC)
[c]NS = not significant $P > 0.10$.

Changes in the Sow's Non-suckled Mammary Glands During Lactation

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At parturition, all mammary glands undergo lactogenesis and glands which are routinely suckled continue to grow (Kim et al., 1999). Piglets begin to develop a preference for specific teats within the first few hours after birth. Once teat order is established, mammary glands which are not regularly suckled begin regressing by a process of involution. Although not producing milk, non-suckled mammary glands may impact the physiology of the sow during lactation. The presence of non-suckled glands may affect the total amount of lactating mammary tissue which develops in suckled glands and, therefore, must be supported by the sow during lactation. Regression of the non-suckled glands should contribute to the pool of endogenous amino acids available for lactation, although the extent of this contribution is not known. In addition, the speed with which the non-suckled glands undergo involution and the type of tissue changes which occur during that involution will determine how long after parturition non-suckled glands may retain lactation function and be available for cross-fostering.

Little is known about the fate of the sow's mammary glands which are not regularly suckled during lactation. The objectives of this study were to characterize the regression of mammary glands which were not regularly suckled during lactation and to determine if this regression is affected by nutrition and litter size. Our hypothesis is that non-suckled mammary glands will undergo regression and this process will be affected by dietary nutrients and litter size.

Data from two different experiments are summarized. In the first experiment, Sixty-nine sows were fed one of four diets representing combinations of two protein levels and two energy levels during lactation (Table 1). Litter size was adjusted to 10 by cross-fostering. Sows were killed either on d 0 (within 12 h postpartum, n=8), 5 (n=9), 10 (n=9), 14 (n=13), 21 (n=15), or 28 (n=15) of lactation. Another group of twenty-eight primiparous sows were allotted to have litter sizes of 6, 7, 8, 9, 10, 11, or 12 pigs (n=4 per group) within 2 d postpartum (Experiment 2) and were killed on d 21 ± 2 of lactation. Mammary glands were collected at slaughter on d 21 of lactation and trimmed of skin and the extraneous fat pad. Individual non-suckled mammary glands were separated, weighed, and ground for measurement of protein, fat, and DNA contents.

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Regression of non-suckled glands mostly occurs during the first week of lactation

Substantial regression of non-suckled mammary glands occurs during the first week to 10-d of lactation, with relatively less further tissue change occurring throughout the remainder of lactation. The change in non-suckled gland wet weight indicates that tissue mass is reduced by about two-thirds during this early period of lactation. The rate of regression of non-suckled mammary glands during lactation is affected ($P < 0.05$) by dietary nutrient level. Tissue wet weight and protein content are affected ($P < 0.05$) by dietary energy level. Mammary dry weight and fat content are affected ($P < 0.05$) by both dietary energy and protein. Rate of regression during early lactation is slower ($P < 0.05$) for sows receiving a high-energy high-protein diet than for those receiving a low-energy low-protein diet, as indicated by loss of tissue wet weight, fat content, and protein content. Tissue wet weight and weights of tissue components are maintained at a higher level ($P < 0.05$) during later lactation in sows receiving a high-energy high-protein diet compared with sows receiving a low-energy low-protein diet. The decrease in tissue DNA amount, an indicator of cell loss, is slower ($P < 0.05$) for sows receiving high-energy diets than for those receiving low-energy diets. By d 21 of lactation, differences in tissue components are not apparent in comparisons among litter sizes.

Conclusions

Regression of non-suckled mammary glands in the lactating sow occurs rapidly over the first week of lactation. Attempts to increase litter size by cross-fostering pigs after the first several days after farrowing generally is unsuccessful because of the loss of lactation function of the tissue and the irreversibility of the involution process. Rate of regression in non-suckled glands is affected by the dietary energy and protein levels which sows received during lactation. Rate of regression of non-suckled glands is generally slowest under dietary conditions where growth of suckled glands is fastest. Enhancing milk production and rate of growth of suckled glands by maximizing dietary nutrient intake during lactation also results in maintenance of greater mass of tissue in the non-suckled mammary glands.

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Table 1. Composition of experimental diets (Experiment 1)

	LELP[1]	LEHP[1]	HELP[1]	HEHP[1]
Chemical composition :				
Energy (Mcal ME/kg)	3.00	3.00	3.50	3.50
Crude protein (%)	15.60	26.50	12.10	22.90
Lysine (%)	0.80	1.62	0.64	1.30
Met+cys (%)	0.54	0.97	0.47	0.78
Threonine (%)	0.57	1.16	0.46	0.93
Valine (%)	0.80	1.62	0.64	1.30
Calcium (%)	0.90	0.85	0.85	0.85
Phosphorus (%)	0.70	0.70	0.70	0.70
Maximum Daily nutrients allowance :				
Diet (kg/day)	4	4	5	5
Energy (Mcal ME/day)	12	12	17.5	17.5
Crude protein (g/day)	624	1060	605	1145
Lysine (g/day)	32	65	32	65

[1] LE: low energy, HE: high energy, LP: low protein, HP: high protein.

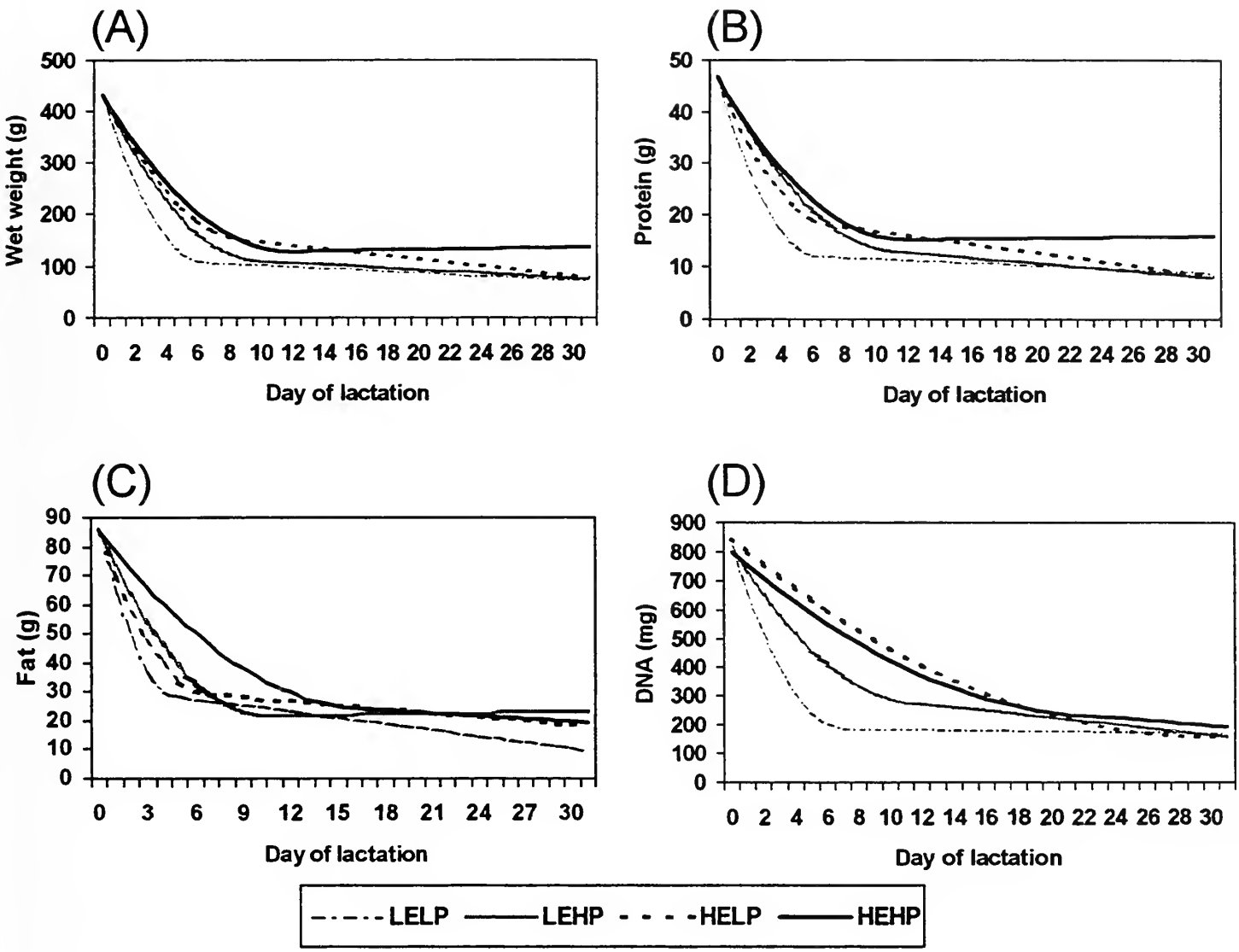

Figure 1. Regression of non-suckled mammary glands. Sows received different nutrient levels during lactation. (A) Wet weight, (B) Protein, (C) Fat, and (D) DNA. LELP, low-energy low-protein; LEHP, low-energy high-protein; HELP, high-energy low-protein; and HEHP, high-energy high-protein.

Changes in the sow's mammary gland after weaning

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Introduction

Mammary gland involution in sows after weaning is an important part of the normal cycle of mammary growth, lactation, and involution. The process of mammary gland involution is initiated at cessation of milk removal, which results in regression of lactation function. In swine, cessation of milk removal occurs the day the piglets are weaned. The act of weaning initiates a process of active tissue involution that involves both functional and structural changes in the mammary gland. Mammary gland involution often is characterized by substantial histological changes in mammary tissue, changes in mammary gland secretions, and loss of lactating epithelial cells. There is a general lack of information available about the process of mammary gland involution in swine.

By day eight postweaning the sow's mammary glands contain little secretion (Cross et al., 1958). Changes in mammary gland secretion composition which indicate a decrease in tissue metabolism are complete by day three to four postweaning (Atwood and Hartman, 1995). Recent work in our laboratory used mammary tissue composition to characterize the regression of non-suckled mammary glands during lactation. The mass and composition of the non-suckled glands are significantly regressed by days five to ten of lactation (Kim et al., 2000).

The objectives of this study were to characterize the compositional changes in porcine mammary tissue that occur during the transition from the lactating state to the nonlactating state. We hypothesize that the mass of the porcine mammary gland will decrease with time after weaning.

Experimental Approach

Animals and Experimental Design. Primiparous gilts (purchased from the Pig Improvement Company, Franklin, KY) were bred and housed at the University of Illinois Swine Research Center (Champaign, IL) and were managed with standard procedures. Sows were weighed at weaning and on the day of slaughter. Teat order relative to suckling behavior was observed the day prior to weaning to determine which mammary glands piglets suckled. Sows were weaned at d 21 of lactation (d 0 of involution). Sows were randomly assigned to treatment groups based on day postweaning. Sows were killed on day 0 (day of weaning; n = 5), or d 2, 3, 4, 5, or 7 after weaning (n = 4 for each day). The protocol for this experiment was approved by the Laboratory Animal Care Committee of the University of Illinois at Urbana-Champaign. Animals were available for monitoring by the Institutional Veterinarian of a representative.

Tissue Collection. Sows were transported to the University of Illinois Meat Science Laboratory at 0600 prior to the morning feeding for slaughter. The animals were electronically stunned and killed by exsanguination. Suckled and non-suckled teats were identified as determined prior to weaning. All mammary glands were removed from the body. Skin and extraneous fat pad were trimmed. Parenchymal tissue from individual glands was dissected and each gland was weighed. Half of each gland parenchyma tissue was ground in a commercial blender (Waring Products, New Hartford, CT) and stored at -20_C for chemical analysis (Kim et al., 1999).

Chemical Analysis. Frozen ground tissue was used for measuring dry matter content, total tissue protein content, and crude fat content. Dry matter content of tissue was measured by desiccation at 105_C for 8 hours. Crude fat content was determined by Soxhlet extraction using a chloroform:methanol (87:13) binary extracting solution with previously dried tissue. Tissue protein content was obtained by measuring nitrogen content by the Kjedahl method.

Statistical Analysis. Data were analyzed only from glands known to have been suckled during lactation. Statistical analysis of the data were performed using the General Linear Model procedure in SAS/STAT® software.

Results

The wet and dry weight of involuting porcine mammary glands decreased over the first seven days postweaning ($P < .0001$; Table 1). Additionally, both the protein and the fat were significantly decreased over the first seven days postweaning ($P < .0001$; Table 1).

Table 1. Postweaning change in mammary tissue components weights.

	Days Postweeaning					
Tissue Component	0	2	3	4	5	7
Wet tissue (g)	485.29 _ 6.9[1]	316.48 _ 7.9	304.82 _ 7.8	248.65 _8.3	201.05 _ 7.8	152.25 _ 8.0
Dry Tissue (g)	100.62 _ 0.4	82.62 _ 0.5	82.77 _ 0.5	58.63 _ 0.5	47.68 _ 0.5	37.44 _ 0.5
Protein (g)	50.94 _ 2.5	34.96 _ 2.8	30.23 _ 2.9	24.67 _ 3.0	21.71 _ 2.8	16.58 _ 2.8
Fat (g)	46.23 _ 4.0	45.08 _ 4.4	44.71 _ 4.7	27.67 _ 4.7	27.14 _ 4.5	20.77 _ 4.4

[1] Least squares means of component _ standard error.

Mammary parenchymal wet weight decreases by about two-thirds during the initial 7 d postweaning. Additionally the dry mammary tissue decreases by over 60% in the first 7 d postweaning. The protein in the mammary tissue decreases by almost 70%, and the fat in the gland decreases by almost 80% during the initial 7 d postweaning. This rapid change in the mammary gland may affect future lactation performance. The changes seen in the gland prepare the gland for the growth that will occur during the next pregnancy.

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"Dynamic Ideal Protein": A novel approach to feeding lactating sows

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The key to establishing nutrient requirements for lactating sows is not limited to maximizing milk yield for nursing pigs, but extends also to maintaining optimum body condition for the subsequent parities (Noblet et al., 1990; Pettigrew et al., 1992a; 1992b; NRC, 1998).

Modern sows are leaner and more productive than those used 20 yr ago (MLC, 1979; 1999). However, modern sows have a low appetite as a result of selection for leanness (Kanis, 1990). Thus, modern sows are often in a catabolic state during lactation due to a high demand of nutrients for milk production and as a result of inadequate feed intake especially in primiparous sows. Excessive tissue loss during lactation is one of the important reasons for reproductive failure of sows during subsequent parities (Reese et al., 1982a; 1982b; Jones and Stahley, 1995). Litter size is an essential factor to consider when establishing nutrient requirements for lactating sows. As litter size increases, sows need increased amounts of nutrients for the maintenance and growth of lactating mammary glands (Kim et al., 1999) and for increased milk production (King, 1991; Whittemore, 1993). When dietary nutrient supply does not meet the amount required to support large litters, tissue mobilization occurs (Kim and Easter, 2000).

To minimize excess tissue mobilization of lactating sows with a low appetite, lactation diets should be designed to provide nutrients with maximum efficiency. Thus, knowledge of which nutrients are limiting for supporting maintenance and production of lactating sows is essential to achieving this goal. Limiting essential amino acids can be predicted by considering the balance between output (i.e., milk amino acids and amino acids used for mammary gland growth) and input (i.e., amino acids mobilized from the tissues).

The objective of our study was to characterize amino acid mobilization among body tissues of the sow and to determine the order of limiting amino acids and ideal amino acid pattern for primiparous sows during lactation

Twenty-eight primiparous sows (Camborough-15, Pig Improvement Company, Lexington, KY) were used and allotted to have litter sizes of 6, 7, 8, 9, 10, 11, or 12 pigs (n=4)

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within 2 d postpartum. Sows were allowed to consume a restricted amount of diet (85% of nutrients for the maximum mammary gland growth as previously reported from Kim et al., 1999b) during lactation. This was done so that clear responses in nutrient mobilization from body tissues would occur as litter size increased. Sows were killed on d 21 of lactation and the carcass, liver, gastrointestinal tract, reproductive tract, mammary gland, and other viscera were separated, weighed, ground, and analyzed for dry matter, crude protein, and amino acids. Simple linear equations were obtained for each amino acid within tissues as a function of litter size.

Approach

From simple linear equations, the amount of each amino acids additionally mobilized as litter size increased by one pig was obtained (Factor 1). In the meanwhile, the amount of each amino acids additionally secreted as a milk protein and additionally used for mammary gland growth and maintenance as litter size increased by one pig were obtained as well (Factor 2).

Diet should provide adequate amino acids to balance out the differences between Factor 1 and Factor 2. However, the amino acid profiles of Factor 1 and Factor 2 are not identical. Thus, dietary amino acid profile should be adjusted according to the levels of Factor 1 and Factor 2.

Amino acids in the milk and used for mammary glands (Factor 1) are relatively fixed components which means that the milk production is rather 'demand driven' by the litter. Sows trying to provide adequate milk to the litter until they reach a limit imposed by the nutrients for milk production. Within this limitation, the level of tissue mobilization can change depending on milk production and feed intake of the sows. Thus, dietary amino acid profile should be adjusted according to the level of tissue mobilization. This is the concept of 'Dynamic Ideal Protein' as a feeding strategy for lactating sows.

Limiting amino acids changes depending on the level of tissue protein mobilization

The amount of essential amino acids obtained from the difference between the amount mobilized and the amount secreted as a milk or used for mammary gland is the amount that should be compensated from dietary amino acids and we believe this represents the ideal amino acid pattern, an ever-changing or "dynamic" value.

Based on assumption that sows are consuming a common corn-soybean meal based lactation diet (71.1% corn and 22.8% soybean meal), using true ileal digestibility (Stein et al. 1998), threonine was shown to be the first limiting amino acid, equal to (or followed by) lysine for our first-parity lactating sows that were experiencing significant tissue mobilization (Table 1).

In a situation where sows are experiencing minimal body weight losses, a different order of amino acid limitation is likely. As tissue mobilization becomes less important, the amino acid profile in milk and mammary gland tissue becomes a major factor influencing the ideal amino acid pattern for lactating sows. As tissue mobilization level was reduced from the 100% level in our study to 0% (no mobilization), valine becomes more limiting than threonine for lactating sows (Table 1).

Conclusions

The body condition and expected level of amino acid mobilization are important factors that must be considered in designed diets for lactating sows. The ideal amino acid pattern for lactating sows is dynamic and depends on the expected body weight loss of sows during lactation. We believe that applying 'dynamic ideal protein' can provide a valid basis for estimating the amino acid needs of lactating sows under specific herd circumstances. For sows having a low appetite during lactation, threonine is a critical amino acid, whereas valine becomes increasingly important for sows having a high appetite during lactation. Lysine is the principal limiting amino acid in the both cases.

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Table 1. Ideal amino acid pattern and the order of limiting amino acids as tissue mobilization level from a sow differs during lactation

	Level of tissue protein mobilization				
	100%	80%	40%	10%	0%
	Ideal amino acid pattern relative to lysine				
Lysine	100	100	100	100	100
Threonine	75	69	63	60	59
Valine	78	78	78	77	77
Leucine	128	123	118	115	115
Isoleucine	60	59	59	59	59
Phenylalanine	57	57	56	56	56
Phe+Tyr	130	123	114	110	109
Arginine	22	38	59	69	72
Histidine	34	36	38	38	39
	Order of limiting amino acids				
First limiting	Threonine	Lysine	Lysine	Lysine	Lysine
Second limiting	Lysine	Threonine	Threonine	Valine	Valine
Third limiting	Valine	Valine	Valine	Threonine	Threonine
Fourth limiting	Phe + Tyr	Isoleucine	Isoleucine	Isoleucine	Isoleucine
Fifth limiting	Isoleucine	Phe + Tyr	Histidine	Histidine	Histidine

Use of α-1,6-galactosidase and β-1,4-mannanase improves the ileal digestibility of energy and amino acids in pig diets.

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Soybean meal is a major protein source for swine and other monogastric animals. However, soybeans, like other legume seeds, contain anti-nutritional factors. Scientists have been successful in eliminating the principle growth inhibitors in soybeans through physical and chemical processing. However, galactosyl oligosaccharides, i.e., α-galactosides and β-galactomannan, known as flatulence-producing factors are still present in soybean meal produced by conventional processes (Rackis, 1981). The content of α-galactosides (raffinose, 1.0% and stachyose, 4.6%) and β-galactomannan (1.2%) is relatively high in soybean meals and these α-galactosides and β-galactomannan are not digestible by pigs and other monogastric animals because of the lack of nzymes that target α-1,6- and β-1,4-galactosyl bonds. Consequently, the levels of indigestible galactosyl oligosaccharides are negatively related to energy and protein digestibility and growth in swine (Veldman et al., 1993; Gdala et al., 1997) and poultry (Coon et al., 1990).

One possible approach to alleviating the anti-nutritional effects of α-galactosides and β-galactomannan in soybean meals is through the dietary inclusion of appropriate enzymes, i.e., α-1,6-galactosidase and β-1,4-mannanase. If effective, the utilization of α-galactosides and β-galactomannan should increase the energy available to the pig. The objective of this study was to test a hypothesis that dietary addition of an enzyme cocktail composed of α-1,6-galactosidase and β-1,4-mannanase to soybean meal based nursery and grow-finisher diets improves energy and amino acid digestibility.

The carbohydrase mixture used in this study was composed of α-1,6-galactosidase (50 Unit/g, Unit is equal to 1 μmol pNPG hydrolyzed within 1 min at 30 °C and pH4.0) and β-1,4-mannanase (110 Units/g, Unit is equal to 1 mg total reducing sugars-glucose equivalent-released within 1 min at 30 °C and pH 4.0) as major active ingredients (40% by weight). These carbohydrases were obtained from the fermentation of *Aspergillus niger* and *Aspergillus orizae*. The carrier for the enzyme mixture was dehydrated barley malt sprouts, 60% of the carbohydrase premix by weight.

In the first experiment, ten nursery pigs (d 48 of age, 16.8 ± 0.7 kg), each fitted with a cannula in the terminal ileum (Giesting and Easter, 1991; Kim and Easter, 2001) were allotted to two treatments. Pigs were fed either the control diet (a common phase III diet, University of Illinois) or the treatment diet (control diet + 0.25% carbohydrase). Pigs were assigned to treatment group on the basis of weight. Experimental diets were provided based on body weight

(daily feed allowance = 0.09 x $BW^{0.75}$). Diets were provided three time per day (0700, 1300, 1900 hrs). There was a 5 d adjustment phase and a 3 d collection phase. In the second experiment, eight ileal-cannulated finishing pigs (93.9 ± 2.0 kg) were allotted to treatments in two, 4 × 4 Latin squares. Treatment 1 to 3 were formed using a base diet that contained 35% soybean meal as the sole protein source. Diet 1 contained by supplemental enzyme and 0.05% and 0.10% total enzyme was added to form diets 2 and 3, respectively. All diets were designed to have 17% CP except for diet 4 which contained casein (4.4%) and was used to measure endogenous protein loss. This approach is based on the assumption that casein is fully digestible. Pigs were fed twice a day (0800 and 1800 h). The daily ration was a fixed amount within a replicate for the 5-d adaptation period and the subsequent 2-d collection. The amount of feed during each subsequent period was adjusted on the basis of 0.09 $kg^{.75}$ of the average body weight of pigs within the replication.

α-1,6-galactosidase and β-1,4-mannanase improved ileal digestibility of energy and major amino acids in soybean meal based diet

In the first experiment, pigs fed diets containing 0.25% carbohydrase derived more ($P <$ 0.01) apparent ileal digestible gross energy than when fed the control diet. There was a 7% improvement in energy digestibility. Apparent ileal digestibilities of major limiting essential amino acids, i.e., lysine, threonine, tryptophan, were greater ($P < 0.05$) when carbohydrase was supplemented (0.05%) than not. Total amino acid content was more ($P < 0.05$) digestible when carbohydarse was supplemented in a young pig diet (Table 1).

In the second experiment, ileal digestibility of gross energy was greater ($P < 0.05$) when enzyme was supplemented to soybean meal based diet at the 0.05% level (Table 2). There was a three percent improvement in both apparent and true energy digestibility. Supplementing enzyme at the 0.10% level did not improve energy digestibility. Addition of either 0.05% or 0.10% enzyme improved ($P < 0.05$) both apparent and true ileal digestibility of major limiting essential amino acids, i.e., lysine, threonine, and tryptophan (Table 2). There was no improvement in ileal digestibility in methionine and branched chain amino acids with enzyme supplementation.

Conclusions

This study provides some evidence that exogenous α-1,6-galactosidase and β-1,4-mannanase may improve energy extraction from soybean meal by both nursery and finisher pigs. It is troubling that 0.05% enzyme improved energy digestibility while a greater concentration did not. However, ileal sampling is not a widely accepted method for assessment of energy digestibility. Ileal digestibilities of major amino acids in soybean meals were improved by the addition of α-1,6-galactosidase and β-1,4-mannanase as well. Elimination of flatulence-producing factors maybe related to improvement of amino acid digestibility. It is still remaining to measure the disappearance of flatulence-producing factors in the ileal digesta.

Acknowledgment

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Table 1. Apparent ileal digestibility of energy and major amino acids in phase III nursery diet.

	Control	Carbohydrase (0.25%)	SEM	$P <$
Gross energy	64.4[b]	68.6[a]	0.79	0.01
Lysine	80.0[b]	81.0[a]	0.28	0.05
Threonine	67.9[b]	71.6[a]	0.88	0.05
Tryptophan	80.0[b]	83.1[a]	0.74	0.05
Methionine	84.5	85.3	0.45	NS
Cysteine	59.7[b]	67.6[a]	1.81	0.05
Valine	77.0	76.4	0.38	NS
Leucine	79.6	81.0	0.89	NS
Isoleucine	78.0	78.0	0.83	NS
Histidine	80.0[b]	81.5[a]	0.33	0.01
Amino acids, avg	76.2[b]	78.4[a]	0.41	0.05

[a, b] Within a row, means lacking a common superscript letter differ.

Table 2. Apparent ileal digestibility of energy and major amino acids in soybean meals based finisher diet

	Control	Carbohydrase (0.05%)	Carbohydrase (0.10%)	SEM
Gross energy	82.00[a]	84.33[b]	82.38[a]	0.33
Lysine	85.20[a]	86.92[b]	87.32[b]	0.36
Threonine	74.89[a]	77.73[b]	77.79[b]	0.55
Tryptophan	80.82[a]	86.99[b]	86.59[b]	0.84
Methionine	85.75	87.30	87.24	0.33
Cysteine	77.16[a]	80.97[b]	78.30[a]	0.54
Valine	79.14	79.49	80.39	0.59
Leucine	82.96	84.12	84.68	0.55
Isoleucine	80.94	80.86	82.20	0.58
Histidine	85.64[a]	89.11[b]	87.47[ab]	0.54
Amino acids, avg	80.67[a]	83.30[b]	82.36[ab]	0.48

[a, b] Within a row, means lacking a common superscript letter differ ($P < 0.05$).

Association of Total Milk Solids Intake and Litter Growth: Data from α-lactalbumin Transgenic Sows.

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Milk is a pivotal factor in piglet survival, growth, development, and pre-weaning piglet body composition. The demonstration that heavier weaning piglets attain market weight faster than lighter weaning piglets (Mahan and Lepine, 1991) has sparked increasing interest in exploiting the lactation period to enhance overall piglet growth and pork production. With increasing desire for piglets that grow at a faster rate and attain greater lean mass deposition, tailoring milk quantities and milk composition to optimize these traits would be of great value to the swine producer.

In swine, 44% of the variation in preweaning piglet growth is attributed to the variability in milk quantities (34%) and total milk solids content (15 %) produced by the sow (Lewis et al., 1978). The other 56% of variability in preweaning growth is attributed to genetic and environmental factors. Furthermore, sow milk yields and milk composition have been identified previously as key limiting factors to preweaning piglet growth and consequently to all stages of postweaning piglet growth (Boyd and Kensinger, 1998). On average, sow reared piglets grow at a rate of 215 g/d in the first weeks of life (Tritton et al, 1993), whereas piglets reared on a milk replacer diet provided ad libitum can grow at a rate of 500g/d, suggesting that sow reared piglets are not attaining maximal growth rate potential during lactation (Hodge, 1974). Furthermore, by day 10 of lactation, sow milk production becomes limiting to piglet growth rates (Harrell et al, 1993). The goal of our laboratory is to develop approaches that will increase sow milk production and reduce the gap between potential and actual piglet growth rates during lactation.

We have demonstrated that transgenic sows that produce greater quantities of the milk protein, α-lactalbumin (α-LA), produce significantly more milk than non-transgenic sibling controls. Furthermore, piglets reared by these α-LA transgenic sows grow at a significantly faster rate compared to piglets reared by non-transgenic control sows. Differences in piglet weight gain become significant by day 6 or 7 of lactation, and continue to be significantly different throughout the lactation period. By day 21 of lactation, piglets reared by transgenic sows are 13% heavier than piglets reared by control sows. The objectives of the following research are to determine if increased milk quantities produced by transgenic sows are associated with increased total solids intake by piglets and to determine if total solids intake is associated with increased piglet growth.

Total milk solids intake by litters was determined on days 3, 6, 9, and 12 of lactation. Total milk solids intake was determined by measuring the percentage of total solids content in sow milk by gravimetric analysis and calculating total solids intake from

daily milk intake by litters. Daily milk intake was estimated by the weigh-suckle-weigh method.

Total milk solids intake by litters was significantly greater in piglets reared by transgenic sows on days 3 and 6 of lactation (Figure 1; $P < 0.05$). On days 9 and 12 of lactation, total milk solids intake was not different between transgenic reared and control reared piglets. Linear regression analysis was used to determine if total milk solid intake by litters is correlated with litter weight gain (Figure 2). Litter weight gain was adjusted for variability in birth weights. Litter weight gain was positively correlated with total solids intake by litters on day 6 of lactation. There was a statistical trend ($P < 0.1$) for this correlation to be significant on day 6 of lactation with 16% of the variation in litter weight gain associated with total milk solids intake by litters. Litter weight gain was not significantly correlated with total milk solids intake by litters on days 3, 9, or 12 of lactation.

In summary, transgenic reared litters were ingesting more total solids early in lactation as compared to control reared litters. Furthermore, increased litter weight gain tended to be associated with increased total milk solids intake by litters on day 6 of lactation, which was also the time period transgenic reared piglets begin to grow at a significantly greater rate ($P < 0.05$; data not shown). Surprisingly, significant correlations of litter weight gain and total milk solids intake were not present on days 3, 9, or 12 of lactation. This in part may be due to the limited number of observations we were able to obtain in this experiment. Many studies of this nature use animal numbers upward of 100 or greater to obtain significant results.

Our data suggest that if litters ingest greater quantities of milk and total milk solids early in lactation, as is the case with the transgenic reared piglets, this in part may be responsible for establishing a faster rate of gain early in lactation. Our data also suggests that the early increase in weight gain by transgenic reared litters is being maintained throughout the rest of lactation. Considering transgenic reared piglets have greater weaning weights compared to control reared piglets, transgenic reared piglets should have greater postweaning performance compared to control reared piglets. This could have significant implications for improved efficiency of swine production, but further research on postweaning performance needs to be conducted to confirm this hypothesis.

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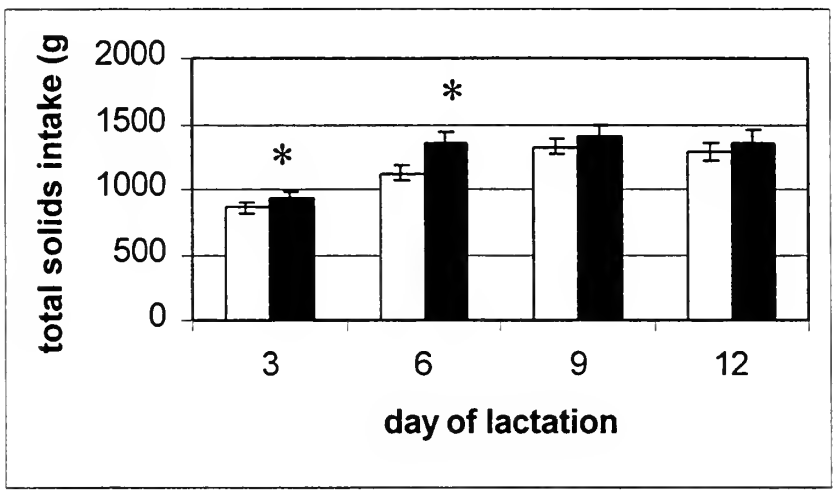

Figure 1. Total milk solids intake by transgenic reared and control reared litters during lactation (N = 12). Transgenic reared litters are represente by the hatched bars, and control reared litters are represented by the white bar *P < 0.05.

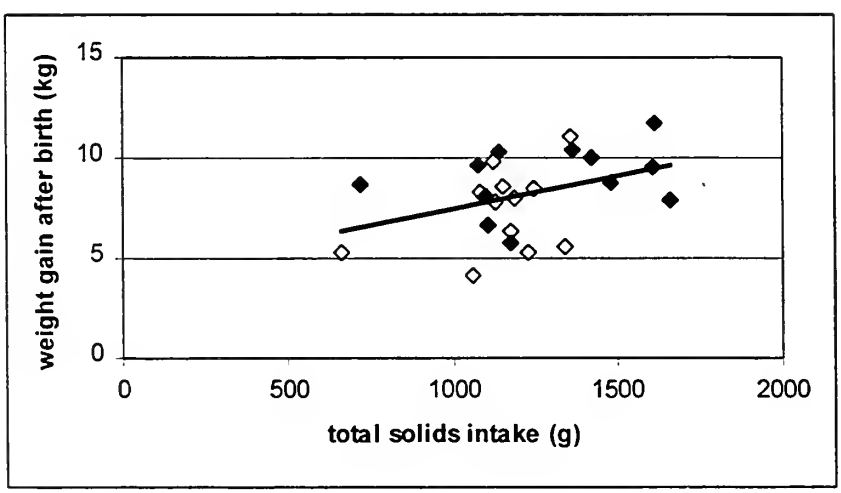

Figure 2. Correlation between preweaning litter weight gain after birth and total milk solids intake by litters (N = 24). Transgenic reared litters are represented by the solid diamonds and control reared litters are represented by the open diamonds. $r^2 = 0.16$; $P < 0.1$.

Effect of Group Size on Pig Performance in a Wean-to-Finish Production System[1]

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Introduction

The "wean-to-finish" production system is being advocated largely because of claims of improved animal performance and a reduction in labor needed for animal movement compared to conventional two- or three-stage systems. However, there has been little, if any, research carried out with this system and consequently limited research data are available to evaluate such claims or to provide an objective basis for developing the optimum design and management approach for a wean-to-finish system.

A major issue is the optimum group size to use in wean-to-finish systems. Large group sizes (≥100 pigs) have been advocated on the basis of reduced facility costs, but there is concern that growth rate may decrease in large groups of pigs (Wolter et al., 2000a). A number of studies have shown a negative relationship between group size and growth (Kornegay and Notter, 1984). There is, however, evidence that the impact of group size on growth varies with size of pig and may be greater in nursery and growing pigs (< 50 kg live weight) (Hyun, 1997; Verdoes et al. 1998). The objective of this research was to determine the effect of group size on pig performance in a wean-to-finish facility.

Materials and Methods

The experiment was conducted to evaluate three group sizes (25, 50, and 100 pigs per pen) and was carried out at the Burton Russell Swine Research Farm, United Feeds, Inc., Frankfort, IN. A total of eight replicates of crossbred (Bunge Line 5 × Bunge Line 13), weaned pigs (17 d of age) were used. After weaning, pigs were randomly allotted to treatment pens on the basis of sex and weight. The ratio of barrows to gilts was constant across treatments within each replicate.

Pigs were housed in an insulated, tunnel-ventilated, wean-to-finish house with concrete slatted flooring and were given ad-libitum access to feed. The dietary regimen consisted of ten-phases and each was formulated to meet or exceed NRC (1998) nutrient requirements. Diets were fed according to a budget allowing each pen of pigs to consume a similar quantity per pigs of each dietary phase. Pen dimensions (length × width) were 5.74 × 3.05 m for 25-pig groups. Partitions

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were removed, increasing pen width to 6.10 m and 12.20 m for groups of 50 and 100, respectively. The floor space allowance for all treatments was 0.68 m^2/pig. Pens were equipped with a hanging drinker with two nipples per 25 pigs and a three-place feeder per 25 pigs (Jumbo Wean-to Finish Feeder, Farmweld, Teutopolis, IL) that provided a total of 106.1 cm of trough space per feeder. Feeders were accessible in the fenceline for groups of 25 pigs and from both sides for pens of 50 and 100 pigs.

Air temperature was maintained using thermostatically controlled heaters and fan ventilation. The temperature was set at 24°C for wk 1 and 2 and then lowered by 2°C per week until it reached 18°C, where it remained for the rest of the experiment. During wk 1 post-weaning, supplementary heat was provided by propane brooders.

Pigs were weighed individually at the beginning and end of the experiment. The coefficient of variation for each pen was calculated to evaluate variation in pig BW. Pigs were weighed in groups of 25 animals every 4 wk during the experimental period.

A pen of pigs was taken off test when the average pig weight reached 116 ± 2.5 kg. Pigs remained in their experimental groups on a finisher phase diet for 2 to 3 wk after completing the growth phase of the study, and were then transported to a commercial slaughter facility where carcass data were collected. Prior to loading, pen live weights were obtained at the farm. Approximately one hour post-mortem, individual hot-carcass weights were recorded and carcass measures were taken with an ultrasound measuring system with the scans being taken longitudinally and anterior to the last rib, 5.5 cm off the midline.

Pig performance data were analyzed as a randomized block design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The pen was considered the experimental unit. The model included effects of treatment, room, and day of weaning nested within room.

Results and Discussion

Pig weight and variation in weight (as indicated by the coefficient of variation) are given in Table 1. Pigs in groups of 50 and 100 animals compared to 25 were lighter at the end of wk 8, and had lower ADG and G/F, but similar ADFI during the period from weaning to 8 wk after weaning (Table 1). However, during the period from 8 wk to the end of the experimental period (116 kg BW) pigs in groups of 100 compared to 50 animals had greater ADG, with pigs in groups of 25 being intermediate for ADG. Average daily feed intake during this period was similar for all group sizes, however, G/F was greater for groups of 100 compared to 25 and 50 animals. Overall, for the period from weaning to end of test, pigs in all group sizes had similar ADG, ADFI, and G/F. At the end of the experimental period, pig BW and variation in pig BW within a pen (as indicated by the coefficient of variation) were similar across the three group sizes. Finally, mortality rates were similar among group sizes, but morbidity (pigs removed due to poor health or injury) was higher in groups of 25 compared to 50 (Table 1).

Previous research suggest that the effect of group size may be greater in lighter pigs than in heavier animals. For example, European researchers reported lower ADFI and ADG for nursery pigs in groups of 90 and 45 pigs compared to 10, but pigs in the larger groups grew faster than

those in the smaller group during the grow-finish period (Verdoes et al., 1998). Similarly, Spoolder et al. (1999) also found a lower ADG in growing pigs (35 to 65 kg BW) kept in larger groups (80 compared to 40 or 20 pigs/pen), whereas, during the finishing period (65 to 85 kg BW), pigs in the three group sizes all had similar levels of performance. Petherick et al. (1989) observed a lower ADG for growing pigs (20 to 60 kg BW) in groups of 36 pigs/pen compared to 8 or 16. In contrast, McGlone and Newby (1994) found no effect of group size (10 vs 20 vs 40 pigs/pen) during the combined grow-finish period (23 to 95 kg BW). In earlier studies conducted in Illinois with smaller group sizes, Gehlbach et al. (1966) and Randolph et al. (1981) reported that group size (4 vs 6 vs 8 and 5 vs 20 pigs per pen, respectively) did not affect pig performance during the finishing period. However, Gonyou and Stricklin (1998) found ADFI and ADG were reduced with increasing group size (i.e., 3, 5, 6, 7, 10, and 15 pigs/pen) during both the growing and finishing phases (25 to 97 kg BW).

Recent research conducted at this center with nursery pigs has shown a negative effect on ADG of increasing group size from 20 to 100 animals (Wolter et al., 2000a; 200b). In these nursery studies, there was no effect of group size on G/F, suggesting that the reduced weight gain resulted from a reduction in ADFI. An attempt to improve the access to feeders by providing multiple feeding locations compared to a single location within a pen did not increase ADFI or ADG in the large groups (Wolter et al. 2000b). In the current study, pigs housed in the wean-to-finish building in larger groups (i.e. 50 and 100 compared to 25) had poorer growth rates during the first 8 wk of the study. However, there were no differences in feed intake found among group sizes, therefore, pigs in the small group size had a greater feed efficiency for this period.

A potential explanation for the differential effect of increasing group size on growth in lighter and heavier pigs comes from research conducted at the University of Illinois that compared group sizes of 2, 4, 8, and 12 pigs during both the growing (26 to 48 kg BW) and finishing periods (84 to 112 kg BW) (Hyun, 1997). Result of this research indicated that finishing pigs changed feeding behavior and maintained ADFI and ADG as group size increased, however, growing pigs showed limited changes in feeding behavior and, consequently, reduced ADFI and ADG with increasing group size (Hyun, 1997).

The finding in this study that morbidity was greatest in the groups of 25 pigs is unexpected. Research conducted by McGlone and Newby (1994) found that pig injury and morbidity were greatest for groups of 40 compared to 10 and 20 pigs per pen. Randolph et al. (1981) found that the total number of aggressive encounters between pigs increased with increases in group size, and Spoolder et al. (1999) observed that number of aggressive interactions at the feeder and number of skin lesions increased with number of pigs per pen. In the current study, pigs were observed daily by trained personnel and there were no signs of excessive skin abrasions on pigs for any treatment at any point during the study period that could have indicated high levels of aggression. The increase in morbidity in 25-pig groups may have been due to chance, and requires further investigation.

Carcass measures taken post mortem indicated no difference among group-size treatments for carcass yield, backfat, and loin-eye depth measurements, or for predicted carcass lean percentage (Table 2). Therefore, the effect of group size on carcass characteristics appears limited.

Implications

These results indicate that pig producers using wean-to-finish facilities may pen pigs from weaning to market in groups of 25 to 100 pigs without impacting performance or carcass measures.

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Table 1. Effect of group size on BW, variation in BW within a pen, growth performance, mortality and morbidity of pigs reared in a wean-to-finish system

Item	Group Size			Avg. SEM	Level of Significance[a]
	25	50	100		
No. of pens	8	8	8	-	-
Weight, kg					
Start of test	5.9	5.9	5.9	0.02	NS
End of wk 8	34.8[b]	33.9[c]	33.9[c]	0.15	***
End of test	116.4	116.1	116.2	0.94	NS
CV, %[d]					
Start	10.7	11.1	11.1	0.14	NS
End of test	9.4	9.6	9.8	0.45	NS
Days on test	168	167	166	0.8	NS
Growth performance					
Start to wk 8					
Daily feed intake, g	815	818	821	9.0	NS
Daily gain, g	512[b]	499[c]	498[c]	3.6	**
Gain:feed	0.63[b]	0.61[c]	0.61[c]	0.004	**
Wk 8 to end					
Daily feed intake, g	2232	2206	2231	15.0	NS
Daily gain, g	716[bc]	708[c]	733[b]	5.9	**
Gain:feed	0.32[c]	0.32[c]	0.33[b]	0.003	**
Start to end					
Daily feed intake, g	1759	1755	1759	13.2	NS
Daily gain, g	655	648	658	4.7	NS
Gain:feed	0.37	0.37	0.37	0.002	NS
Mortality, %[e]	3.0	1.5	1.0	-	NS
Morbidity, %[e]	7.0[b]	3.5[c]	3.9[bc]	-	*

[a] NS, *, **, *** = not significant, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.
[b,c] Means within the same row with unlike superscript letters differ.
[d] CV = coefficient of variation, values determined from pig weights within each pen.
[e] Means compared using the Kruskal-Wallis rank-based test.

Table 2. Effect of group size on carcass ultrasound measurements of pigs reared in a wean-to-finish system

Item	Group Size			Avg. SEM	Level of Significance[a]
	25	50	100		
No. of pens	8	8	8	-	-
No. of pigs[f]	175	367	740	-	-
Slaughter, and carcass					
Body weight, kg	126.9	125.4	125.9	0.88	NS
Hot carcass weight, kg	95.0	94.5	94.9	0.89	NS
Backfat[b], mm	21.5	21.0	20.6	0.38	NS
Loin depth[b], mm	62.9	64.2	63.6	0.71	NS
Percent lean[c], %	53.4	53.7	53.8	0.17	NS
Dressing percent, %	74.9	75.3	75.4	0.35	NS

[a] NS,* = not significant, $P < 0.05$, respectively.

[b] Measures were taken longitudinally and anterior to the last rib 5.5 cm off the midline using ultrasound.

[c] Predicted from backfat and loin-depth measures (J. Stouffer, personal communication).

[d, e] Means within the same row with different superscripts differ.

[f] Pigs weighing less than 95 kg BW were removed before slaughter.

Effect of hut design on farrowing and lactation performance of pigs housed in a hoop structure.

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Introduction

Economics drive profitability in hog production units. Inputs such as buildings and equipment are the second leading expense behind feed costs. Hoop structures offer a low cost alternative form of swine housing, however, production levels must be evaluated in any new system.

Published data for group housed sows during farrowing and lactation suggests mortality rates of 16-19% (Goetz and Troxler, 1995; Gustafsson, 1983; Cronin and Smith, 1987). These levels are much higher than those reported in modern confinement systems where sows are crated for the entire farrowing and lactation period. Modern farrow-to-wean farms strive to keep their pre-weaning mortality below 10% of live born piglets. The rates published in many group housed farrowing situations are at least double that of commercially acceptable levels. The historical move of sows from pasture systems (a group farrowing situation) to an indoor farrowing crate system stemmed from this problem of high preweaning mortality. It was discovered that restricting sow movements led to reduced crushing and, thus, the farrowing crate was borne. Today, the majority of sows are confined in crates for farrowing and lactation.

No published research is available on sow and piglet performance during farrowing and lactation in a hoop structure and, thus, the focus of this study was to evaluate performance parameters associated with different farrowing hut designs in such a system.

Materials and Methods

Animals in this study were bred and reared at the Moorman Swine Research Farm at the University of Illinois in Urbana-Champaign. Forty-eight primparous and multiparous crossbred sows were mated to Duroc sires and introduced as a group to the treatment pens within the hoop structure at approximately day 107 of gestation. Performance of the piglets was measured during a 21 day lactation period.

Equipment. Two different designs of hoop structure were used in this study (Span-tech, Houghten, Iowa and Coverall, Saskatoon, SK). Both buildings were 30ft wide by 84 ft long and were constructed of polyethylene fiber canvass stretched over a roof formed with metal pipes and had a 4 foot high wood sidewall. A removable door made of the same polyethylene canvass was present on both ends of the structure to facilitate natural ventilation. A concrete pad extended 20 ft into the building on the south end and a feeder (eight hole, Farmweld) was anchored in the

center of the concrete pad. Two heated waterers (four hole, Mirafount) were situated on the concrete in each building. Outdoor wooden (Smidley, Marting Manufaturing, Iowa) and galvanized metal (Portahut, Storm Lake, Iowa) farrowing huts were used for the farrowing experiment. A roller door was designed to keep piglets inside for the first 7 to 14 days of life. The device was constructed of a smooth metal or PVC plastic cylinder mounted on a frame which was bolted to the door of the hut. The roller protects the sow's udder as she passes into and out of the hut.

Trial Design. Forty-eight sows and their litters were used to evaluate the performance effects of two farrowing hut designs in a 2 X 2 factorial arrangement. Sows were selected and balanced across treatments for breed and parity. The sows were allotted to their respective treatments one week before farrowing. The treatment group had access to huts equipped with roller doors designed to keep piglets inside the hut for 10 to 14 days while allowing the sow free movement into and out of the hut. The control group had huts that had no roller door and, thus, the piglets were free to move out of the hut immediately after birth. The sows had access to six huts from one treatment group throughout the farrowing and lactation period. Sows were allowed free choice of hut but were moved if they farrowed in the aisle or on the concrete. Some females were locked in a hut during farrowing to prevent them from scattering their litters. During the extreme hot weather, farrowing sows were drenched by hand with buckets of cool water and a 4.5 ft portable fan with a mister was installed at the front of the hoop structure. The fan ran from 1200h to 2200h each day. The four groups of sows averaged 6 days between the farrowing dates for the first and last litter in each group and the piglets were processed after the last litter was born. The sows were removed from the building during processing to preserve the safety of the technician and piglets. The sows were allowed ad libitum access to a lactation diet that met or exceeded NRC (1998) recommendations. A creep area (2.8m x 5.1m) equipped with creep pellets was available for piglets from day 5 post-farrowing onwards.

Performance Measurements. Piglets were weighed at the time of processing and again at weaning. Number born per sow, number born alive, and number of mummies was recorded. Mortalities were recorded each day for each sow.

Results

The results of this study are presented in Table 1. Hut design did not influence the number of live piglets born per sow (13.1 vs. 12.0 piglets, for huts with roller and without roller, respectively, SE = 0.66, $P > 0.05$) or the number weaned per sow (5.3 vs. 5.1 piglets, for huts with roller and without roller, respectively, SE = 0.81, $P > 0.05$). High levels of mortality (60% of liveborn piglets) occurred within the hoop structure system. Hut design did not affect ($P > 0.05$) mortality rate. Piglets reared in the hut without a door roller had higher individual piglet weaning weights compared to those having a roller in the door (4.5 vs. 4.2 kg BW, for huts with roller and without roller, respectively, SE = 0.09, $P < 0.02$). Lighter weaning weights may have been due to the piglets in the huts with roller bars having reduced access to the sow relative to the piglets housed in huts without rollers. It is possible that the sow controlled of nursing frequency in the treatment group, whereas, the piglets controlled this behavior in the huts without roller

doors, as they displayed more frequent udder stimulation. In order to fully explore this theory, a behavior study has been conducted and is in the process of being analyzed.

In summary, huts fitted with a roller on the door resulted in lighter piglet weaning weights, but did not reduce piglet mortality within a hoop structure system.

Conclusions

1. Mortality rates in this loose-housed system for farrowing and lactation were extremely high.
2. Hut design did not affect piglet mortality.
3. Installing a roller door in the entrance to the hut resulted in reduced piglet weaning weights compared to huts with no roller door, but had no effect on mortality.

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Table 1. The main effects of roller doors in farrowing huts on litter performance in a hoop structure environment.

	Roller type		SE	P value
Trait	R	NR		
Number of observations	24	24		
Number of piglets per litter:				
Born	13.1	12.0	0.66	0.06
Stillborn	1.1	.84	0.45	0.51
Weaned	5.3	5.1	0.81	0.76
Litter weight, kg				
Start[1]	21.7	21.7		
Weaning[1]	24.2	28.1	4.2	0.28
Total litter gain[1]	2.42	6.41	4.2	0.28
Survivability, %[2]	40	40	0.09	0.99

[1] Corrected to a litter start weight of 21.7 kg/sow using covariance analysis.
[2] Survivability = [Number weaned/(Number born - Number stillborn) x 100]

Effect of Frequency of Boar Exposure on Estrus and Ovulation in Weaned Sows as Determined by Real-Time Ultrasound.

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Introduction

Reproductive performance in swine is most related to inseminations occurring near the time of ovulation. This is due to the time-span for normal fertilization of eggs being limited by both the egg after the occurrence of ovulation (<12 h) and the sperm after insemination (<24 h). Therefore, the number of eggs that are fertilized, the percentage of normal embryos, the resulting pregnancy rates (Hunter and Dzuik, 1968, Soede et al., 1995), farrowing rates (Nissen et al., 1997), and litter size (Kemp and Soede, 1996; Rozeboom et al., 1996) are all related to time of insemination relative to ovulation. Insemination within 12 h before ovulation, appears to produce the most optimal pregnancy rates (>90%) and litter sizes (10.5-11, Kemp and Soede, 1996) but Nissen et al has observed that inseminations occurring from 28 h before to 4 h after ovulation result in high reproductive rates (Nissen et al., 1997). Unfortunately, the time of ovulation is difficult to predict since the only obvious marker for this event is the expression of standing estrus. Further, the time of ovulation varies between 24 to 60 h after onset of estrus (Soede et al., 1992). This high level of variation is important since insemination protocols are set from estrus, with inseminations typically occurring twice between 0 and 48 h. There appears to be a normal distribution for ovulation time after onset of estrus, with 20% of weaned sows ovulating by 24 h, 55% ovulating between 24 and 48 h, and 25% ovulating after 48 h (Knox et al., 1999a). It would appear then, that current insemination protocols may not fully account for the total variation in ovulation times within the breeding herd. This would help explain why average USA reproductive rates are less than optimal.

This experiment was conducted to address whether estrous detection frequency could be used as a method to increase the frequency of fixed time inseminations that occur within 24 or 12 h prior to the time of ovulation in anticipation of early (≤ 24 h), expected (≥24 h and ≤48 h) and late times of ovulation (≥ 48 h). In commercial settings, a common industry practice employs a once daily estrous detection procedure with insemination targeted to occur at 0 h for the first service (estimated time relative to estrus is 0-24 h) and 24 h later for the second service (estimated time relative to estrus is 24-48 h). Although this procedure can generate 79% farrowing rates, it cannot account for the variation in time of ovulation, since onset of estrus can be off by as much as 24 h. Even twice daily estrous detection procedures (0800 h and 1600 h) that rely on an 8 h working day, cannot compensate for this variation, since sows detected in estrus at 0800 h, may have already been in estrus for up to 16 h. Additionally, since approximately 70% of the females will show the first standing response between 6 p.m. and 6 a.m., large estimation errors result (±16 h) for onset of estrus, leading to inseminations that fail to occur within 24 h prior to ovulation.

This experiment was designed to evaluate the effect of increasing frequency of boar exposure for accurately determining onset of estrus and time of ovulation in weaned sows. More frequent estrous detection was evaluated for its effect on percent of sows returning to estrus and

ovulating, interval from weaning to estrus, interval from estrus to ovulation, frequency of ovulation, and percent of inseminations occurring within 24 h prior to the time of ovulation.

Materials and Methods

The experiment was conducted in six replicates between January and August 2000. Sows were from parities 1 to 9 (average parity 3.9 ± 0.2), and represented commercial maternal-line genotypes, and both crossbred and purebred maternal and terminal lines from 3 different farms. Sows were weaned on average 18.2 ± 0.3 d after parturition (range: 14 to 30 days). Sows were randomly allotted to boar exposure frequency of once daily (1X, n = 63), twice daily every 12 h (2X, n = 61), or three times daily at 8 h intervals (3X, n = 62) by genotype, parity, and lactation length. Sows were weaned into gestation crates with boars housed in separate rooms or at least 40 feet away. Boar exposure was initiated at 1300 h 3 days after weaning with exposure to boars occurring in the alleyway at the front of the crate for approximately 2 to 5 minutes. Estrus was determined using the back-pressure test. Once standing estrus was observed, transrectal ultrasound was performed every 8 h to visualize the ovaries and determine the occurrence of ovulation. All sows were artificially inseminated twice with 3.0 billion sperm cells in long-term extender and used ≤ 4 days from collection. Sows in the 1X group were bred at 0 and 24 h, sows in the 2X group at 12 and 24 h, and sows in the 3X group at 16 and 32 h after first detected in estrus.

Results

The wean to estrus interval was not influenced frequency of boar exposure and averaged 4.5 days (Table 1, Figure 1). The percent of sows expressing estrus in 8 days (90-100%) was not influenced by treatment or month (Table 2). However, the percentage of sows ovulating tended to be influenced by treatment ($P > .05$) with 84.5% of 2X and 3X boar exposed sows ovulating compared to 98% of 1X exposed sows (Table 2). The wean to ovulation interval was not influenced by treatment and averaged 6.5 days (149.8 ± 5.1 h). Average largest follicle size at estrus was also not influenced by treatment or month and averaged 7.6 mm (Table 1). Length of estrus averaged 58.5 h and was influenced by treatment and also by month but there was no interaction (Figure 1). Estrus was longer ($P < .005$) in the 3X exposure group compared to the 2X and the 1X exposure groups (Table 1). The shortest length of estrus ($P < .05$) occurred in the months of August, February and March (40 to 49 h), and the longest lengths in April and June (75 to 58 h, Figure 1). The estrus to ovulation interval was not influenced by treatment (Table 1) or month (Figure 1) and averaged 45 h. The occurrence of ovulation to the end of standing estrus was also influenced by month, but not by treatment (Table 1). The shortest interval from ovulation to end of estrus occurred in the February and March replicates (9 to 13 h), and intermediate intervals in late March and August (23 h) and longest intervals in April and June (44 to 33 h, Figure 1). More frequent boar exposure improved the percentage of 1st and 2nd inseminations occurring within 24 h before ovulation (Table 3).

Conclusions

The results of this experiment indicate that increasing frequency of boar exposure for weaned sows can improve the precision for timing inseminations and increase the frequency of 1st and 2nd services administered within 24 h before ovulation. Higher frequency of boar exposure does not influence wean to estrus, estrus to ovulation or wean to ovulation intervals but does increase

length of estrus and could indicate a tendency for lowered return to estrus and ovulation in certain seasons of the year. Season of the year appears to influence both the length of estrus the interval from ovulation to end of estrus. Taken as a whole, it is not clear whether the observed advantage in insemination timing will result in higher farrowing rates and litter sizes and that these will off set the cots associated with lowered estrus and ovulation rates and the extra labor for estrous detection. Lastly, it appears from our results that timing two inseminations to occur within 12 to 24 h before ovulation based off of weaning alone could prove beneficial since ovulation occurs at 149.9 h plus or minus 5 h (145 to 155 h). Future experiments will investigate whether fixed time inseminations that occur at 138 and 150 h after weaning which allow time for sperm capacitation and sperm reservoirs to become established can produce good reproductive performance.

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Table 1. Influence of Frequency of Boar Exposure on Wean to Estrus Interval (WEI), Estrus to Ovulation (EstOv), Wean to Ovulation (WOv), Length of Estrus (LEst), and Follicle Size at Estrus.

	Probability		Boar Exposure Frequency			
Parameter	**Treatment**	**Replicate**	**1X**	**2X**	**3X**	**S.E.**
WEI (h)	NS	NS	114.5	113.9	106.5	5.0
EstOv (h)	NS	NS	38.4	43.1	42.4	5.2
WOv (h)	NS	NS	149.9	152.7	146.9	5.1
LEst (h)	.005	.05	41.7	52.9	62.3	5.3
OvEst (h)	NS	.001	27.5	20.2	24.9	3.9
Follsz (mm)	NS	NS	7.83	7.66	8.00	0.23

Table 2. Influence of Frequency of Boar Exposure Frequency on Percentage of Weaned Sows Expressing Estrus and Percent Ovulating in 8 Days.

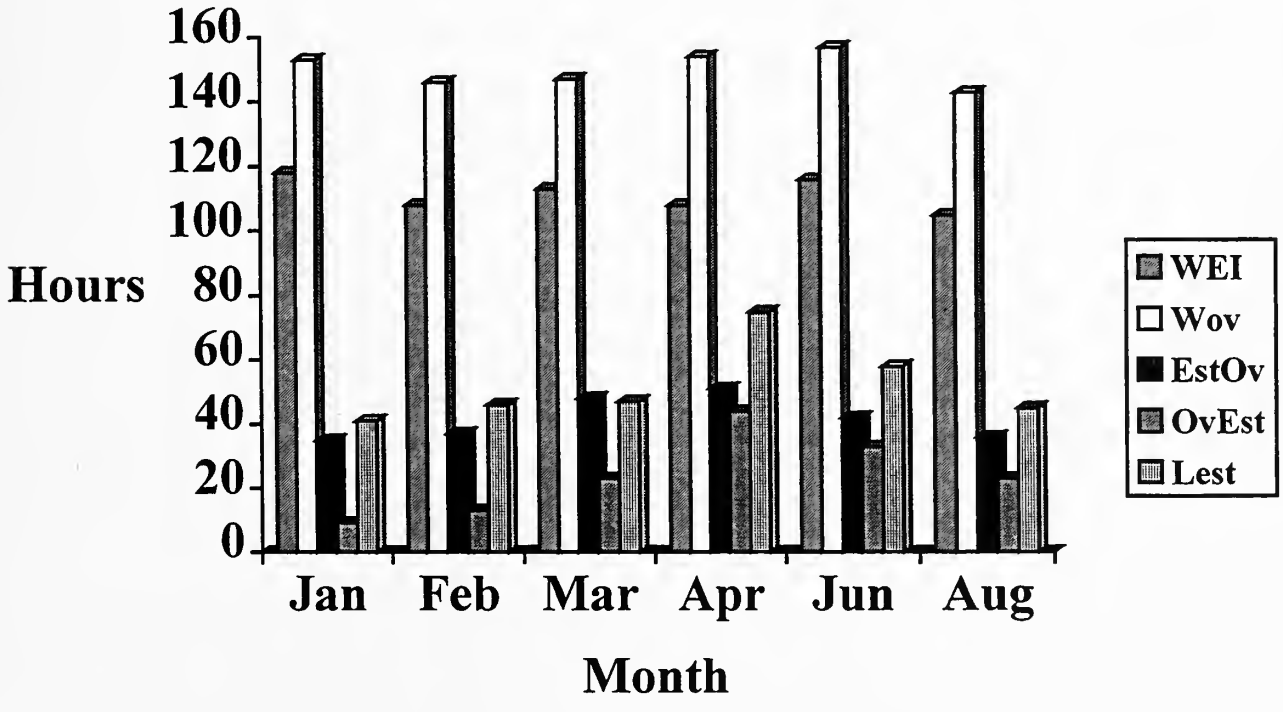
Parameter	**1X**	**2X**	**3X**	**P**
Estrus (%)	98	92	90	NS
Ovulation (%)	98	84	85	NS

Table 3. Influence of Frequency of Boar Exposure Frequency on Percentage of 1st and 2nd Inseminations Occurring at Various Hour Intervals Before or after Ovulation in Weaned Sows.

	Boar Exposure Frequency					
	1X		2X		3X	
Interval	1st (%)	2nd (%)	1st (%)	2nd (%)	1st (%)	2nd (%)
≥-72 h	4.8	0.0	3.9	0.0	7.6	0.0
-48-71 h	19.3	1.6	3.9	0.0	1.9	7.6
-36-47 h	45.1	9.6	17.6	1.9	19.2	1.9
-24-35 h	11.2	12.9	33.3	19.6	17.3	3.8
-12-23 h	17.7	48.3	27.4	33.3	42.3	32.6
0-11 h	1.6	22.5	13.7	39.2	9.6	42.3
+1-12 h	0.0	4.8	0.0	1.9	0.0	9.6
1st A.I. within 24 h (%)	19.3[a]		41.0[b]		51.9[b]	
2nd A.I. within 24 h (%)	70.8[a]		72.5[a]		74.9[b]	

means in row with different superscripts differ, P <.05.

Figure 1. Influence of Month on Wean to estrus (WEI), Wean to Ovulation (WOv), Onset of Estrus to Ovulation (EstOv), Ovulation to End of Estrus (OvEst), and Length of Estrus (LEst).



The Effects of Phytase on Growth Performance, Carcass Characteristics, and Bone Mineralization of Late-Finishing Pigs Fed Corn-Soybean Meal Diets Containing No Supplemental Phosphorus, Zinc, Copper and Manganese

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Abstract
Late finishing swine (n = 408) with an average initial weight of 84 kg were used to evaluate the effects of supplementing microbial phytase to corn-soybean meal diets in which sources of supplemental inorganic phosphorus (P), zinc (Zn), copper (Cu), and manganese (Mn) were removed. The dietary treatments employed were: A) positive control which included a standard trace-mineral premix and inorganic P supplementation (0.16% available P, 0.46% Ca); B) negative control in which a trace-mineral premix without Zn, Cu, and Mn was fed, and no inorganic P was added (0.06% available P, 0.46% Ca); and the negative-control diet supplemented with either C) 300 U/kg microbial phytase; or D) 500 U/kg microbial phytase. With the exception of available P, Zn, Mn and Cu, diets were formulated to meet or exceed NRC (1998) nutrient recommendations for late-finishing pigs. Each supplementation regimen was fed to six pens of 17 pigs (triplicate replicates of both of barrows and gilts) from 84 to 123 kg body weight. Reducing inorganic P and selected trace minerals, or the addition of either 300 or 500 U/kg phytase to the negative control diet did not affect ($P > 0.10$) weight gain, feed intake, or feed efficiency (gain/feed). Moreover, carcass characteristics were not affected ($P > 0.10$) by any of the supplementation strategies employed in this experiment. Metacarpal III and IV bone ash (g), however, was reduced ($P < 0.05$) for pigs fed the negative control diet compared to the fully-supplemented positive-control diet and the diets containing added phytase. These data indicate that supplemental levels of inorganic P, Zn, Cu, and Mn can be reduced or eliminated without deleteriously affecting growth performance and carcass characteristics of late-finishing swine.

Introduction
The late-finishing phase (80 to 120 kg) of swine production accounts for not only the largest amount of feed consumed, but also the lowest feed efficiency compared to the other phases of growth. Nutrient requirements (per unit of diet) for phosphorus (P), trace minerals, and vitamins decrease with increasing age and body weight (NRC, 1998). The current NRC (1998) trace mineral and P recommendations for late-finishing swine have been largely extrapolated from previous research conducted on pigs that were slaughtered at lighter weights than is currently practiced. These requirements are therefore not well established, and may represent overestimates for late-finishing swine. Overfeeding of P and trace minerals leads to excessive excretion and consequent environmental concerns. With this in perspective, the removal of supplemental sources of inorganic P (Pi) and several trace minerals may serve to reduce environmental concerns and lower feed costs.

Supplementation of swine and poultry diets with exogenous phytase sources, such as that produced by *Aspergillus niger*, has consistently been shown to markedly improve phytate-P utilization (Cromwell *et al.*, 1993; Simons *et al.*, 1990), but also zinc (Zn) and manganese (Mn) utilization as well (Lei *et al.*, 1993; Adeola *et al.*, 1995; Biehl *et al.*, 1995). Whether microbial phytase improves copper (Cu) utilization is not clear (Adeola, 1995; Aoyagi and Baker, 1995), but regardless, the Cu provided in corn-soybean meal diets exceeds the NRC (1998) requirement for late-finishing pigs. The objective of this experiment was to evaluate the effects of two levels of microbial phytase on growth performance, carcass characteristics, and bone mineralization of late-finishing swine fed diets in which Pi, Zn, Cu, and Mn supplementation was eliminated.

Materials and Methods

A total of 408 late-finishing pigs (Bunge Line 5 × Bunge Line 13) with an average initial body weight of 84 kg were used to evaluate the effects of supplementing microbial phytase to corn-soybean meal diets in which sources of supplemental Pi, Zn, Cu, and Mn had been removed. Growth performance, carcass characteristics, and bone mineralization were evaluated. The experiment was conducted at a commercial research facility (United Feeds, Inc., Frankfort, IN) in an environmentally-controlled building. The building contained 12 pens per room, and two rooms were used. The three pens of barrows per treatment (12 pens) were housed in one room and the three pens of gilts per treatment (12 pens) were housed in the other room. Pigs were alotted on the basis of sex and initial body weight to pens, after which pens were randomly assigned to one of four dietary treatments. There were three pens of barrows and three pens of gilts for each treatment. There were 17 pigs per pen, and pen served as the experimental unit.

The experiment was conducted using a corn-soybean meal diet (0.66% digestible lysine, 0.06% available P, and 0.43% Ca, cf. Table 1). Dietary treatments consisted of: A) a positive-control diet which included standard trace-mineral premix and inorganic P supplementation (0.16% available P); B) a negative-control diet in which a trace-mineral premix containing no sources of Zn, Cu, or Mn was fed and no Pi was added (0.06% available P); and the negative control diet supplemented with either C) 300 or D) 500 U/kg microbial phytase (Natuphos®). With the exception of available P, Zn, and Mn, all diets met or exceeded NRC (1998) nutrient recommendations. Calculated levels of dietary Cu were in excess of the NRC (1998) requirement for all diets.

The microbial phytase product (Natuphos®) was obtained from BASF Corporation (Parsippany, NJ). The phytase premix was assayed and found to contain 2,338 U of phytase activity/g, with 1 U of phytase activity defined as the quantity of enzyme required to produce 1 μmol of Pi/min from 5.1 mmol/L of sodium phytate at a pH of 5.5 and a water bath temperature of 37° C. Each of the four diets was fed to six pens of 17 pigs (three pens of barrows and three of gilts) from 84 to 123 kg body weight.

Performance data were analyzed on an equal time basis (i.e., the time at which the first pen of pigs reached an average body weight of 123 kg); carcass characteristics and bone responses were analyzed on an equal-BW basis. Therefore, time served as the endpoint for analysis of performance data, and BW served as the endpoint for analysis of carcass and bone data. Thus, when the first pen reached an average body weight of 123 kg (50 d of feeding), pigs in all pens

were weighed and performance criteria were evaluated. Additionally, at that time ultrasound was performed for determination of backfat (mm), loin depth (mm), and percent lean for the heaviest pen of pigs. Based on the previously determined average daily gain and number of days to reach 123 kg BW, each additional pen was weighed and ultrasound scanning was performed at the appropriate time. Upon achieving their target final body weight of 123 $\pm$ 3 kg, the three median-weight pigs from each pen were killed for collection of metacarpal III and IV bones. Body weight within sex at the time of ultrasound scanning and bone collection was used as a covariate for analysis of treatment differences in carcass characteristics and bone response criteria. Bones from individual pigs were pooled by pen and subsequently dried, after which they were ashed for determination of metacarpal III and IV ash weight and percent ash.

Results

Growth performance, carcass characteristics, and bone ash results are shown in Table 2. Regardless of treatment imposed, no differences ($P < 0.10$) in growth performance or carcass characteristics were observed. Removal of Pi and selected trace minerals in the absence of phytase depressed ($P < 0.05$) metacarpal III and IV bone ash (g) when compared to that of the positive-control treatment, although no differences were observed for dry bone weight and bone ash (%). Phytase addition to the negative-control diet resulted in increased ($P < 0.05$) dry bone weight and bone ash (g), and these measures of P adequacy did not differ ($P > 0.10$) from those of pigs fed the positive-control diet. Although no differences in bone ash (%) were observed, removal of supplemental Pi, Zn, Mn and Cu resulted in both total bone mass and subsequent bone ash (g) reductions ($P < 0.05$) when phytase was not present in the diet. These data indicate that removal of supplemental Pi and selected trace minerals from the finishing diet for 50 d did not deleteriously affect growth performance or carcass characteristics, but did reduce bone weight and ash weight without affecting percent ash. Furthermore, dietary addition of phytase ameliorated these reductions in bone response criteria, and responses to 300 U/kg supplemental phytase were equal ($P > 0.10$) to those of 500 U/kg supplemental phytase.

Discussion

The most logical means of ameliorating environmental concerns associated with large-scale livestock enterprises is to simply reduce the dietary inputs of trace elements, P, and nitrogen. This, in turn, decreases the amount of these nutrients available for excretion in feces and urine, and also provides the ancillary benefit of reducing feed costs for producers. The heightened environmental concerns of consumers in areas of large-scale swine production also have resulted in increased research efforts to further enhance environmental stewardship among swine producers.

Poultry producers often omit vitamin and (or) trace-mineral premixes during the last week before slaughter as a method of reducing dietary costs and reducing nutrient excretion. Omission of vitamin and trace-mineral premixes from broiler diets for up to three weeks pre-slaughter has been shown to have no detrimental effects on growth performance or carcass characteristics (Skinner *et al.*, 1992b; Deyhim and Teeter, 1993; Christmas *et al.*, 1995). Moreover, removal of supplemental Ca and Pi from broiler diets for one week pre-slaughter also has been shown to not negatively influence broiler performance or carcass characteristics (Skinner *et al.*, 1992a; Chen and Moran, 1994).

Our data indicate that Pi and selected trace mineral supplementation (Zn, Cu, and Mn) can be reduced or eliminated for the last 50 d prior to slaughter without adversely affecting growth performance or carcass characteristics. Bone strength may be impaired, however, as evidenced by reduced bone weight (g) and bone ash (g) in our experiment, but the addition 300 or 500 U/kg phytase ameliorated these depressions. Several hip and leg fractures did occur during normal handling and transportation in our experiment, but this occurred for pigs on all treatments and was not limited to pigs fed the diets with reduced supplementation regimens. Nevertheless, the reductions in bone weight (g) and bone ash (g) do serve to illustrate a primary concern pertaining to the complete removal of supplemental Pi sources during the late-finishing phase. Lindemann *et al.* (1995) investigated the effects supplemental Pi omission length on growth performance and bone strength of finishing pigs. They reported that eliminating supplemental Pi did not adversely affect bone strength in finishing pigs when omitted from 64 to 104-kg body weight, but reduced bone strength when removed for the entire finishing period (50 to 104 kg). Eliminating supplemental Pi, however, did not affect growth performance, regardless of the length of Pi omission. Similarly, van de Ligt *et al.* (1997) reported reductions in the bone strength of finishing pigs fed diets containing no supplemental Pi from not only 50 to 110-kg, but also 70 to 110-kg body weight compared to pigs fed a Pi-supplemented positive control. No serious problems pertaining to bone strength were encountered in our experiment, however, severe reductions in bone strength can result in an increased incidence of fractures, subsequent financial losses, and reduced economic viability.

Harper *et al.* (1997) conducted two experiments that were directed at reducing Pi supplementation of growing and finishing swine diets. In the first experiment, the supplementation of Pi was reduced to provide 0.10% less available P for growing pigs (20 to 50 kg), and supplemental P was completely eliminated for finishing pigs (50 to 109 kg). The positive control diets contained 0.20% and 0.12% available P for growing and finishing pigs, respectively. For both phases, reducing dietary levels of available P produced depressions in average daily gain and feed intake, but only mildly reduced feed efficiency. Phytase addition (250 or 500 U/kg) to the low-P diets, however, ameliorated these performance depressions, and produced performance measures that were similar to pigs fed the fully-fortified positive-control diets. In the second experiment, they examined the effects of phytase addition (167, 333, and 500 U/kg) to a P-deficient diet compared to diets that were formulated to be marginally P-deficient or P-adequate fed to growing and finishing swine (30 to 70 kg and 70 to 107 kg). Pigs fed the low-P negative-control diet grew slower and less efficiently than those fed diets with supplemental P (0.04 and 0.08% added available P) or phytase. Adding graded levels of phytase or Pi overcame these reductions and increased average daily gain, feed efficiency, and bone strength. Carcass quality, however, was not affected by phytase supplementation or available P level. Moreover, the graded phytase additions produced linear increases in the digestibility of P in both experiments, and therefore reduced P excretion by an estimated 21.5%. These data are difficult to interpret, especially regarding Pi removal for finishing pigs because the reduced performance observed in the growing phase may have confounded the results obtained in the finishing phase. These data, however, do illustrate that reducing Pi supplementation in lighter-weight growing pigs (30 to 70 kg) can deleteriously affect growth performance. This, in turn,

may also adversely affect growth performance when those pigs reach the finishing phase of production.

Mavromichalis *et al.* (1999) conducted three experiments to determine the effects of omitting vitamin and trace mineral premixes, as well as reducing Pi additions in conventional corn-soybean meal diets fed to late-finishing swine. The first experiment revealed no adverse effects on growth performance or carcass characteristics with the omission of vitamin and (or) trace-mineral premixes from late-finishing swine diets. In another experiment, the removal of up to two-thirds of the supplemental Pi did not deleteriously affect growth performance and meat quality, and surprisingly resulted in a slight improvement in average daily gain. Complete omission of supplemental P, however, produced a depression in growth performance. The third experiment revealed that the omission of vitamin and trace-mineral premixes, as well as 2/3 of the supplemental Pi did not hinder the performance of late-finishing swine. A tendency, however, was observed for increased backfat thickness and reduced percent lean for diets containing reduced levels of supplemental P and (or) vitamin and trace-mineral premixes. The experiments of Mavromichalis *et al.* (1999), however, did not evaluate the effects of these dietary omissions on leg weakness or measures of P and Ca utilization such as bone ash weight and percent ash.

McGlone (2000) carried out two experiments to investigate the effects of eliminating supplemental sources of vitamins and minerals (including Ca and P) from late-finishing swine diets during the last 30 d prior to slaughter. In both experiments, elimination of supplemental sources of vitamins, minerals, or both, did not affect growth performance, feed efficiency, or health status of the pigs. In parallel with these results, Kim *et al.* (1997) concluded that the omission of trace mineral and (or) vitamin premixes from corn-soybean meal diets fed to late-finishing pigs (70 to 112 kg) did not adversely affect growth performance, carcass characteristics, or meat quality. In subsequent work from Kansas State University, neither growth performance nor carcass traits were negatively influenced when levels of supplemental Zn, Cu, Mn, and Fe were reduced for both early- and late-finishing swine from 71 to 115-kg body weight (James *et al.*, 2000). These latter two studies did not include dietary treatments in which supplemental Pi had been lowered, or eliminated.

Patience and Gillis (1995; 1996) reported no detrimental effects on weight gain, feed intake, feed efficiency, or carcass characteristics of late-finishing swine fed diets without vitamin or trace-mineral supplementation for the final 3 or 5 weeks prior to marketing. Pre-slaughter omission of the vitamin premix, however, was reported to reduce muscle thiamin concentrations and increase intramuscular lipid content in one experiment (Patience and Gillis, 1996). They concluded that these nutritional strategies could reduce feed costs, but they only recommended this strategy for the last 5 weeks prior to marketing. Potential adverse effects of omitting vitamin premixes from broiler diets also has been reported. Deyhim *et al.* (1996) and Patel *et al.* (1997) concluded that although omission of vitamin premixes from finishing broiler diets did not affect growth performance or carcass characteristics, the omission of vitamin premixes did adversely affect riboflavin and thiamin concentrations in broiler meat. Reducing P, trace mineral, and vitamin supplementation of swine diets may not seriously impair growth performance, but it could reduce meat quality and the nutritional value of pork products. Furthermore, Spurlock *et*

al. (1998) cautioned that the removal of all supplemental vitamins and trace minerals during the finishing phase may impair the pig's ability to overcome immune challenges, and therefore may result in small long-term reductions in growth performance.

In agreement with the results of Spurlock *et al.* (1998), Edmonds and Arentson (2000) observed reduced immune competency when both vitamin and trace-mineral premixes were omitted from non-medicated late-finishing swine diets, and adverse affects on meat quality were also seen, regardless of medication regimen. They conducted two experiments employing the same dietary treatments, but diets were medicated only in the first experiment. The three dietary treatments employed were a positive-control diet containing supplemental sources of both vitamin and trace minerals, and two treatments in which both premixes were omitted for either 6 or 12 weeks prior to slaughter. When diets were medicated in the first experiment, omission of vitamin and trace-mineral premixes did not negatively affect growth performance, loin-eye area, or backfat thickness. In the second experiment, however, omission of vitamin and trace-mineral premixes from the non-medicated diets for 12, but not 6 weeks, prior to slaughter produced depressions in average daily gain and feed intake. No effects on loin-eye area and backfat thickness were observed in either experiment, regardless of the duration of vitamin and trace- mineral removal. It would appear that the reductions in growth performance observed by Edmonds and Arentson (2000) were related to impaired immunocompetency, because growth performance was only reduced in pigs fed non-medicated diets.

Edmonds and Arentson (2000) observed a dramatic reduction in the vitamin E content of loin and ham muscle, and also noted a decrease in the Cu content of ham muscles when vitamin and trace-mineral premixes were omitted for either 6 or 12 weeks. More specifically, the vitamin E content was reduced by greater than 75% in loin muscle, and by greater than 50% in ham muscle. Although growth performance and carcass characteristics were not affected by vitamin and trace-mineral withdrawal from medicated finishing swine diets, reductions in muscle vitamin E content of pigs fed under these dietary conditions present concerns for swine producers as well as consumers. Reductions in the intramuscular vitamin E content of pork products increases the susceptibility of pork to cellular peroxidation, and therefore may decrease the shelf-life of the meat (Mahan, 2000). Thus, the omission of vitamin E from finishing-swine diets not only reduces the nutritional quality of the meat and creates concerns regarding consumer acceptability, but also may negatively influence the profitability of swine enterprises.

Park *et al.* (2000) attempted to alleviate the concerns regarding pork quality when trace minerals and vitamins, and particularly vitamin E, are omitted from late-finishing swine diets. In their experiment, they evaluated the effects of the complete omission of vitamin and trace-mineral premixes from late-finishing swine diets on growth performance and pork quality, but they used a special premix that provided Mg, Fe, and vitamins E and C. No effects on growth performance, carcass characteristics, or meat quality were observed among any of the dietary treatments. Intramuscular vitamin E content, however, was not assessed in this work.

The late-finishing phase (80 to 120 kg) of swine production accounts for the largest percentage of feed consumed, and it also generates the greatest amount of manure and the majority of the subsequent environmental concerns. The data discussed herein support a

hypothesis that vitamin and trace-mineral over-fortification, together with excessive supplementation of Pi during the late-finishing period is not essential for ensuring maximal growth performance. Removal of all supplemental vitamins and trace minerals from non-medicated finishing-swine diets, however, may impair the immune competency of the pig, and may indirectly result in depressed growth performance. Moreover, the most worrisome observation regarding vitamin and trace-mineral omissions from late-finishing swine diets is a reduction in the vitamin E content of pork products (Edmonds and Arentson, 2000). This, in turn, creates both nutritional and economic concerns. The results presented herein, as well as related research, indicate that producers should approach reducing Pi, vitamin, and trace mineral supplementation for late-finishing swine with caution.

With proper phytase supplementation, it may be possible to entirely eliminate supplemental levels of Pi, Zn, Mn and Cu from corn-soybean meal diets for late-finishing pigs. Our data herein wherein nonmedicated diets were fed for the last 50 d of finishing support this suggestion. On the other hand, we do not think it would be wise to delete the remaining vitamins and minerals that are normally added to diets for late-finishing swine. Trace minerals such as Se and I are not bound to phytate, and Fe may not be effectively released from the phytate complex when phytase is supplemented (Biehl *et al.*, 1997). Moreover, if supplemental Pi, whether from dical, monocal or defluor, is eliminated from the diet, this also lowers the dietary Fe substantially.

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Table 1. Percentage composition (as-fed basis) of corn-soybean meal diets fed to late finishing pigs from 84 to 123 kg body weight [1]

	Treatments	
Ingredient	*A*	*B,C,D*
Corn	81.64	81.657
Soybean meal, dehulled	15.60	15.60
Soybean oil	1.00	1.00
Limestone	0.72	1.00
Dicalcium phosphate	0.54	---
Trace-mineral salt mix [2,3]	0.30 [2]	0.283 [3]
Vitamin premix [4]	0.05	0.05
L-Lysine•HCl (FG)	0.15	0.15
Crude protein, %	13.8	13.8
Lysine, %	0.77	0.77
Dig. lysine, %	0.66	0.66
Calcium, %	0.46	0.46
Total phosphorus, %	0.43	0.33
Estimated available phosphorus, %	0.16	0.06
Zinc, mg/kg	108	23
Copper, mg/kg	12	6
Manganese, mg/kg	28	11

[1] Treatment A served as the positive control, treatment B served as the negative control, and treatments C and D were supplemented with 300 and 500 U/kg diet phytase, respectively.

[2] A standard trace-mineral premix was added to treatment A to provide the following (per kilogram of diet): NaCl, 2.5 g; Fe ($FeSO_4•H_2O$, 82 mg; Zn (ZnO), 86 mg; Cu ($CuSO_4•5H_2O$), 6.8 mg; Mn (MnO), 17.5 mg; Se (Na_2SeO_3), 0.26 mg; and I (CaI_2), 0.3 mg.

[3] The same mix as that shown for diet A, but with supplemental Zn, Cu, and Mn removed.

[4] Vitamin premix provided the following (per kilogram of diet): retinyl acetate, 500 μg; cholecalciferol, 4.125 μg; DL-α-tocopheryl acetate, 22 mg; menadione sodium bisulfite, 1.1 mg; vitamin B_{12}, 0.01 mg; riboflavin, 2.2 mg; D-pantothenic acid, 6.05 mg; niacin, 8.25mg; choline, 60.5mg.

Table 2. Effects of supplemental phytase on performance of late-finishing pigs fed corn-soybean meal diets containing no supplemental sources of inorganic phosphorus, zinc, copper, and manganese from 84 to 123 kg body weight

	Treatments				
	A	B	C	D	
Pi, Zn, Cu, Mn [1]	+	-	-	-	
Item / Phytase (U/kg)	0	0	300	500	Pooled SEM
Growth performance [2]					
Gain, g/d	784	770	774	778	11
Feed intake, g/d	2794	2771	2757	2727	34
Gain:feed, g/kg	281	279	281	286	2
Carcass characteristics [3]					
Backfat, mm	18.2	19.0	18.0	18.3	0.4
Loin Depth, mm	59.1	58.7	58.6	59.4	0.5
Lean, %	53.5	53.2	53.5	53.5	0.1
Metacarpal III and IV [3]					
Dry bone weight, g	36.0^{ab}	34.3^{b}	36.5^{a}	36.9^{a}	0.6
Bone ash, g	18.2^{a}	17.1^{b}	18.1^{a}	18.5^{a}	0.3

[1] Inorganic phosphorus, zinc, copper, and manganese supplementation.
[2] Data represent combined means of triplicate groups of both gilts and barrows during a 50-d feeding period; each of the six pens per treatment contained 17 pigs.
[3] Data represent pen-mean values of the three median-weight pigs in each of the six pens.
[a-b] Means within a row not having common superscript letters are significantly different ($P < 0.05$).

MICROBIAL PHYTASE DOES NOT AFFECT PROTEIN-AMINO ACID UTILIZATION IN SOYBEAN MEAL, REGARDLESS OF DIETARY PHOSPHORUS LEVEL

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Overview

The efficacy of supplemental microbial phytase for improving phytate-phosphorus (P) utilization has been investigated extensively, and phytase has been shown to consistently improve phytate-P utilization in both swine and poultry (Simons *et al.*, 1990; Cromwell *et al.*, 1993; Biehl *et al.*, 1995). Improved utilization of dietary phytate-P caused by phytase supplementation offers producers a viable means of reducing inorganic P supplementation, and it also effectively reduces P excretion. Concerns pertaining to nitrogen (N) pollution present another environmental challenge facing swine producers, and microbial phytase may have a role in the liberation and utilization of phytate-bound amino acids. Phytase addition to swine and poultry diets, therefore, may improve not only the utilization of dietary phytate-P but also protein, although research dealing with the latter is inconsistent and controversial (Kornegay, 1996; Kornegay *et al.*, 1998a; 1998b; Ravindran *et al.*, 1999; Boling *et al.*, 1999; Zhang *et al.*, 1999; Peter *et al.*, 2000). Phytase-mediated responses in protein digestibility, if any, are of lower magnitude than responses in phytate-P utilization, and the potential improvements have seldom manifested in improved growth performance. The ability of phytase to not only liberate phytate-bound P, but also improve protein-amino acid utilization would provide swine and poultry producers a means to further aid in the alleviation of social and environmental concerns presently encompassing large-scale livestock production. In addition to reductions in inorganic P supplementation, phytase-mediated increases in protein-amino acid utilization would also enable producers to feed lower-protein diets and reduce N excretion, thereby creating both economic and environmental advantages.

The collective research on phytase-mediated improvements in protein utilization are inconclusive and inconsistent. The inconsistencies may be a result of the wide range of evaluation methods (e.g., amino acid digestibility, N retention, growth performance), protein sources, and levels of calcium and P that have been used to investigate protein utilization as affected by phytase. Investigating the potential efficacy of phytase for improving protein-amino acid utilization has been a focal point for research conducted in our laboratory in the last year. Thus, the research presented herein was conducted to evaluate whether the small phytase-mediated improvements in amino acid digestibility observed by ourselves and others would transmit into noticeable improvements in growth performance, feed efficiency, and protein efficiency ratio (PER, ie., g weight gain per g of protein intake). Research also is presented investigating the potential protein efficacy of phytase as affected by dietary levels of calcium and available P.

Materials and Methods

Nursery Pig Assay. The objective of this experiment was to evaluate the effects of microbial phytase on protein-amino acid utilization in soybean meal (SBM) fed to nursery pigs. Two

levels of CP (12 or 24%) provided by SBM were fed in the absence and presence of 1,200 U/kg of microbial phytase in a 2 × 2 factorial treatment arrangement. Sixty-four nursery pigs (Line 326 sire × C22 dams; PIC, Franklin, KY) with an average initial body weight of 10.6 kg and an average age of 38 d were used in a 17-d growth assay. Following a 12-h period of feed deprivation, pigs were assigned to uniform blocks based on ancestry and body weight. The pigs were then allotted randomly from blocks to pens, and pens were randomly assigned to dietary treatments. There were four pigs per pen and four pens per treatment.

The SBM used in the swine assay was analyzed to contain 47.0% CP (AOAC, 1995), and the levels of SBM inclusion were selected to represent protein levels that were both deficient (12% CP) and adequate (24% CP). A cornstarch-sucrose basal diet that contained 0.74% Ca and 0.33% available phosphorus was used, with SBM and phytase being added at the expense of cornstarch (Table 1). All diets were offered in meal form, and all nutrients, with the exception of protein, met or exceeded NRC (1998) nutrient recommendations. No attempt, however, was made to equalize available P and Ca levels in the experiment. Diets containing 12% CP furnished 0.79% Ca and 0.38% available P, and those containing 24% CP provided 0.89% Ca and 0.44% available P. Thus, the addition of SBM to provide 12 or 24% CP also furnished available P and Ca levels such that these diets were slightly in excess of NRC (1998) requirements for 10 to 20-kg pigs. The ratio of Ca:available P, however, was maintained at approximately 2:1.

The pigs were housed in an environmentally controlled nursery with 100% expanded-metal flooring. Each pen (1.2 m^2) was equipped with a five-hole self-feeder and a nipple waterer to allow ad libitum consumption of feed and water. Temperature at the animal level was maintained at 25° C. Pigs and feeders were weighed at the initiation and termination of the 17-d assay period, and average daily gain, feed intake, CP intake, and feed efficiency (gain/feed) were determined. Weight gain and CP intake were then used to calculate PER values.

Chick Assays 1, 2, and 3. The objective of chick assays 1, 2, and 3 was to use slope-ratio methodology to assess the effects of microbial phytase on the protein quality of SBM fed to broiler chicks consuming diets formulated to contain either adequate or 50% excesses [i.e., above NRC (1994) required levels] of both Ca and available P. A total of 288 female broiler chicks (96 per assay) were fed three levels of CP (5, 10, and 15%) furnished by SBM in the absence and presence of 1,200 U/kg microbial phytase in a 2 × 3 factorial arrangement of treatments in a completely randomized design. The CP levels provided by SBM were selected to represent three amino acid-deficient levels of dietary protein, all of which were first limiting in sulfur amino acids (SAA) as established by Emmert *et al.* (2000). Three independent assays were conducted to evaluate three available P and Ca regimens. The regimens for the respective assays included 1) adequate P and Ca, i.e., 0.45% available P and 1.00% Ca; 2) adequate P and excess Ca, i.e., 0.45% available P and 1.50% Ca; and 3) excess P and Ca, i.e., 0.675% available P and 1.50% Ca. The SBM was analyzed to contain 46.6% CP (AOAC, 1995). The experiment was conducted using a cornstarch:dextrose basal diet (Table 1), with SBM, $CaCO_3$, dicalcium phosphate, and phytase being added at the expense of dextrose to achieve target dietary levels of CP, Ca, and available P. With the exception of protein, all other nutrients met or exceeded NRC (1994) nutrient recommendations. Experimental diets were fed to four replicate pens of four chicks during a 13-d assay period (8 to 21-d posthatching).

Chick Assay 4, 5, and 6. These assays were conducted to further evaluate the effects of microbial phytase on the protein quality of SBM and corn gluten meal (CGM) fed to broilers chicks consuming diets formulated to contain either adequate or 50% excesses of Ca or of both Ca and available P. A total of 288 female broiler chicks (96 per assay) were used in three independent assays evaluating the effects of excess Ca and available P on the efficacy of phytase for improving the PER values of CGM, SBM, and casein. Casein was added to serve as phytate-free positive control protein source. Broiler chicks were fed diets containing exactly 10% CP provided by casein, CGM, or SBM in the absence and presence of 1,200 U/kg phytase.

The potential protein-releasing efficacy of phytase was evaluated using the same three available P and Ca regimens that were used in chick assays 1, 2, and 3. The ingredients were analyzed for crude protein [SBM, 46.6%; CGM, 62.9%; and casein, 88.3% (AOAC, 1995)]. Each of these three experiments was conducted using a cornstarch:dextrose basal diet (Table 1), with SBM, $CaCO_3$, dicalcium phosphate, and phytase being added at the expense of cornstarch:dextrose (2:1, w/w). With the exception of protein, all other nutrients met or exceeded NRC (1994) nutrient recommendations. Experimental diets were fed to four replicate pens of four chicks during a 9-d assay period (8 to 17-d posthatching).

Chick Assay 7. The objective of this assay was to evaluate the effects of microbial phytase on the protein quality of SBM for chicks fed diets containing deficient, adequate, and marginally-excessive levels of available P and Ca provided by inorganic sources or liberated by microbial phytase. Moreover, this assay enabled a clear assessment of protein utilization in SBM fed to chicks as affected by marginal excesses of P provided by inorganic sources or that provided by microbial phytase. The assay consisted of five dietary treatments in a completely randomized design. Diets were formulated to contain 18% CP furnished by SBM. The CP level provided by SBM was selected to represent an amino acid-deficient level of dietary protein which was first limiting in SAA that would still produce substantial growth rates (Emmert *et al.*, 2000). Dietary treatments consisted of a P- and Ca-deficient negative control, which was then supplemented with KH_2PO_4 and $CaCO_3$, or 1,200 U/kg microbial phytase to provide 0.10% available P and 0.05% Ca, and thereby meet NRC (1994) nutrient recommendations. Also, the P- and Ca-adequate diets were supplemented with KH_2PO_4 and $CaCO_3$, or microbial phytase to furnish marginal dietary excesses of available P and Ca, provided by either inorganic sources or by phytate hydrolysis.

The SBM was analyzed to contain 46.6% crude protein and was from an origin similar to the SBM used in chick assays 1 to 6. The experiment was conducted using a cornstarch:dextrose basal diet (Table 1), with SBM, phytase, KH_2PO_4, and $CaCO_3$ being added at the expense of cornstarch. With the exception of protein, available P, and Ca in the negative control diet (diet 1), all other nutrients met or exceeded NRC (1994) nutrient recommendations. Experimental diets were fed to four replicate pens of four chicks during a 12-d assay period (8 to 20-d posthatching). At assay termination, all chicks were euthanized by CO_2 gas and the right tibia was quantitatively removed and pooled by replicate. Tibiae were dried at 110° C for 24 h, weighed, and then dry ashed at 550° C for 24 h in a muffle furnace. Ash weight was calculated as a percentage of dry bone weight, and also as ash weight (mg) per tibia per chick (Chung and Baker, 1990).

Following the respective experimental periods for all chick assays, body weight of individual chicks and group feed intakes were recorded, after which weight gain, feed intake, CP intake, and feed efficiency (gain/feed) were calculated for each replicate pen. Weight gain and CP intake were then used to calculate PER values for each replicate pen and dietary treatment. Independent multiple-linear regression analyses consisting of two straight lines with a common intercept were performed for chick assays 1, 2, and 3 (i.e., within Ca and available P regimen), with weight gain being regressed on CP intake for the three levels of protein furnished by SBM fed in the absence or presence of 1,200 U/kg of phytase. This facilitated use of slope-ratio methodology to evaluate SBM protein utilization as affected by phytase supplementation in the presence of varying levels of Ca and available P.

The microbial phytase product (Natuphos®) used in all experiments was obtained from BASF Corporation (Parsippany, NJ). The phytase premix was assayed and found to contain 805 U of phytase activity/g, with 1 U of phytase activity defined as the quantity of enzyme required to produce 1 μmol of Pi/min from 5.1 mmol/L of sodium phytate at a pH of 5.5 and a water bath temperature of 37° C.

Statistical Analysis

Analysis of variance was performed on pen means data using the General Linear Models (GLM) procedure of SAS® (1990, SAS Inst. Inc., Cary, N.C.) appropriate for a completely randomized design in the chick assays, and a randomized complete-block design in the pig assay. The factorial treatment arrangements of the pig assay and chick assays 4, 5 and 6 were analyzed using single degree-of-freedom orthogonal comparisons. Chick assay 7 was also analyzed using built-in single df comparisons. In chick assays 1, 2, and 3, weight gain was regressed on CP intake, and independent linear regression equations were generated for the three CP levels furnished by SBM fed in the presence or absence of microbial phytase using the GLM procedure of SAS® (1990). The intercepts for the equations generated for the three CP levels fed in the absence and presence of phytase were not different ($P > 0.10$). Therefore, multiple-linear regression analysis consisting of two straight lines with a common intercept was performed using the GLM procedure of SAS® (1990). Increasing CP levels supplied by SBM fed in the presence or absence of microbial phytase were then analyzed using slope-ratio methodology.

Results

Nursery Pig Assay. Feeding nursery pigs amino acid-adequate diets containing 24% CP provided by SBM produced greater ($P < 0.01$) weight gain, feed intake, and feed efficiency, but lower ($P < 0.01$) PER values than those for pigs consuming the amino acid-deficient diets containing 12% CP furnished by SBM (Table 2). Addition of 1,200 U/kg of microbial phytase, however, did not affect ($P > 0.10$) any of the response criteria, regardless of the CP level that was fed. Although phytase addition to the amino acid-deficient diets containing 12% CP numerically depressed average daily gain (344 vs. 320 g/d) and feed intake (798 vs. 722 g/d), numerical improvements in feed efficiency (432 vs. 442 g gain/kg feed intake) and PER (3.60 vs. 3.69) values were observed. Thus, the numerical improvements in efficiency measures resulted from reduced feed intake with the addition of phytase to the amino acid-deficient diet.

Chick Assays 1, 2, and 3. Each of the three chick assays shown in Table 3 were carried out at different times and with a different group of chicks. Thus, comparisons among dietary treatments are valid only within and not between individual assays.

Regardless of the available P and Ca regimen employed, or phytase supplementation, weight gain and feed efficiency (gain/feed) responded linearly ($P < 0.01$) as a function of CP intake for chicks fed graded levels SBM in chick assays 1, 2, and 3 (Table 3). Regressing weight gain (g) on CP intake (g) for Diets 1 to 3 (no phytase, X_1) and Diets 4 to 6 (+ phytase, X_2) resulted in the following common-intercept multiple-linear regression equations for the respective available P and Ca regimens: 1) adequate Ca and P, $Y = - 6.84 + 3.26X_1 + 3.23X_2$, $R^2 = 0.99$; 2) excess Ca, adequate P, $Y = - 4.54 + 3.21X_1 + 3.22X_2$, $R^2 = 0.99$; and 3) excess Ca and P, $Y = - 7.64 + 3.29X_1 + 3.17X_2$, $R^2 = 0.99$. No effect ($P > 0.16$) of phytase supplementation on slope values was observed, regardless of dietary calcium and available P regimen. The results indicated that a 50% excess, i.e., above NRC (1994) required levels, of Ca or both Ca and available P had no effect whatever on the capacity of phytase to affect the utilization of protein or amino acids in SBM. In all assays, phytase supplementation did not ($P > 0.10$) affect protein utilization in SBM as assessed by chick growth responses and PER, although there was a tendency for phytase supplementation to improve PER when 5% CP diets were fed. Other research in our laboratory had shown that the 5% CP SBM diet is first limiting in cystine, whereas the 10% CP and 15% CP SBM diets are first limiting in methionine. All three of the SBM diets used in chick assays 1, 2 and 3 were nonetheless severely deficient in SAA.

Chick Assays 4, 5, and 6. As was the case for chick assays 1, 2, and 3, the three chick assays depicted in Table 4 were conducted at different times with different groups of chicks. Treatment comparisons, therefore, are valid only within individual assays. The SBM used in these three assays (and in chick assays 1, 2, and 3), however, was out of the same batch.

A 50% excess, i.e., above NRC (1994) required levels of Ca or of both Ca and available P had no effect ($P > 0.10$) whatever on the capacity of phytase to affect protein utilization in CGM or SBM fed to young chicks (Table 4). As expected, growth performance and PER values were greater ($P < 0.01$) for SBM than for casein, and they were greater ($P < 0.01$) for both SBM and casein than for CGM. Surprisingly, phytase addition tended to reduce the PER of casein in diets containing 0.45% available P and 1.00% Ca. Conversely, phytase addition tended to increase 9-d weight gain and PER values of casein for chicks fed diets containing 50% excesses of both available P and Ca. These responses, however, were observed for a phytate-free protein source, and therefore are not entirely understood. Nonetheless, microbial phytase did not improve growth performance or PER of chicks fed diets containing Ca and available P levels that were at or in excess of NRC (1994) requirements.

Chick Assay 7. This assay was analyzed statistically based on the assumption that provision of 0.10% inorganic P (Pi) plus 0.05% Ca was equivalent to provision of 1,200 U/kg of phytase (Table 5). Thus, after testing "basal" vs "supplemented," i.e., "deficient" vs "adequate" (diet 1 vs diets 2 to 5), one could evaluate diets 2 to 5 as a 2 x 2 factorial arrangement of treatments.

The basal diet (diet 1) containing 18% CP from SBM together with 0.95% Ca and 0.35% available P was designed to be deficient in protein and methionine, and marginally deficient in

Ca and available P (NRC, 1994). Chicks fed this diet responded to P supplementation in that weight gain was increased ($P < 0.07$) as was both gain/feed and PER ($P < 0.05$). That P addition, whether from KH_2PO_4 or from phytase-induced release of Pi, increased chick performance indicated that the improvements in bird growth indices were caused by P supplementation. Moreover, that Pi addition resulted in performance responses that were not different ($P > 0.10$) from responses due to phytase supplementation indicated that the responses observed were due to P addition alone, and not due to improved protein utilization.

Discussion

Positive effects of microbial phytase on protein and amino acid digestibilities in avians are typified by the experiments of Kornegay (1996), Yi *et al.* (1996a), Kornegay *et al.* (1998b), and Ravindran *et al.* (1999; 2000). The improvements in protein and amino acid digestibility were small in magnitude (1 to 3%), and statistically significant improvements were not always detectable. Thus, some researchers either observed a range of improvements for certain amino acids (Sebastian *et al.*, 1997; Johnston and Southern, 2000a), or simply were not able to detect these phytase-mediated improvements in chicks (Biehl and Baker, 1997a). Moreover, other investigators reported no beneficial effects of phytase on amino acid digestibility in chicks (Zhang *et al.*, 1999).

The inconsistencies in amino acid digestibility responses not only have surfaced in chicks, but also have been observed in pigs. In several experiments, phytase has been shown to improve amino acid digestibility and measures of N utilization in pigs (Officer and Batterham, 1993; Mroz *et al.*, 1994; Kemme *et al.*, 1995; Li *et al.*, 1998; Kornegay *et al.*, 1998a). In a similar manner to responses observed in chicks, other researchers observed either variable responses to phytase supplementation (Yi *et al.*, 1996b; Johnston and Southern, 2000b) or observed no effect of supplemental phytase on amino acid digestibility, or protein utilization per se, in pigs (Murry *et al.*, 1997; O'Quinn *et al.*, 1997; Rodehutschord, 1998; Valaja *et al.*, 1998).

Phytase-mediated improvements in amino acid digestibility measures are relevant, but these improvements, if any, are irrelevant to swine and poultry producers if they do not manifest in enhancements in the more important economic traits (i.e., growth performance, feed efficiency, and PER). Our data clearly illustrate that regardless of possible protein or amino acid digestibility responses, improvements in weight gain, feed efficiency, and PER do not occur when phytase is added to Met-deficient SBM for chicks and pigs.

Many intriguing and perplexing questions arise when evaluating the potential efficacy of microbial phytase for improving protein utilization. If phytase does truly improve protein and(or) amino acid digestibility, why don't these improvements also consistently manifest in improvements in growth performance for nonruminant animals fed amino acid-deficient diets? Moreover, there are concerns pertaining to the levels of dietary Ca and available P which have been used to assess the potential protein sparing effect of phytase.

Some researchers and industry officials have alleged that in order to observe the potential "protein sparing" effect of phytase, one must evaluate the protein efficacy of phytase in swine and poultry diets that are deficient in P. There are concerns, however, pertaining to proper

interpretation of these data obtained when feeding P-deficient diets. Thus, do the potential phytase-mediated improvements result from increased P utilization, improved protein utilization, or a combination of both? What levels of Ca and available P should be used to assess the effects of microbial phytase on protein utilization? In our opinion, the only way to clearly assess the efficacy of phytase for improving protein utilization is to use diets that are solely deficient in the nutrient in question (i.e., protein, amino acids).

The first issue that needs to be addressed is the efficacy of phytase for improving the amino acid digestibility of diets containing adequate levels of available P. The addition of phytase to P-adequate diets has been shown to elicit small positive nitrogen and amino acid digestibility responses in chicks (Kornegay, 1996; Sebastian *et al.*, 1997; Kornegay *et al.*, 1998b; Johnston and Southern, 2000b; Ravindran *et al.*, 1999; 2000), poults (Yi *et al.*, 1996a), and pigs (Officer and Batterham, 1993; Murry *et al.*, 1997; Kornegay *et al.*, 1998a; Johnston and Southern, 2000b). Johnston and Southern (2000b) observed greater ileal amino acid digestibility responses in finishing pigs when 500 U/kg of phytase was added to P-adequate corn-SBM diets than when added to P-deficient corn-SBM diets. In fact, amino acid digestibility responses did not occur when P-deficient diets were fed, but they did occur when P-adequate diets were fed. How can one argue that it is acceptable to evaluate phytase effects on protein or amino acid digestibility in animals fed P-adequate diets, whereas it is unacceptable to evaluate protein utilization via growth assay (PER or slope ratio) under similar assay conditions of P adequacy? Clearly, it is gratuitous to conclude that P-adequate protein-quality assay systems are acceptable when positive responses occur but are unacceptable when no (or negative) responses occur. The levels of available P used in our assays should not have impaired the ability of phytase to potentiate improvements in amino acid digestibility.

What effects, if any, do inorganic P (Pi) and Ca levels exert on protein utilization and potential phytase-mediated increases in amino acid digestibility? Increasing dietary levels of available P (from inorganic sources) from 0.16 to 0.48% has been shown to reduce intestinal phytase activity in chicks (Davies *et al.*, 1970). Mahajan and Dua (1997) also observed an *in vitro* inhibitory effect of Pi on the intrinsic phytase activity of rapeseed. Our assays were conducted with young chickens and pigs. Thus, the possible contribution of intestinal phytase activity, as well as the potential inhibitory effect of Pi levels on intestinal phytase activity, should have been negligible in our experiments (cf. Waldroup *et al.*, 1967; Pointillart *et al.*, 1984; Yi and Kornegay, 1996; Biehl and Baker, 1997b). Moreover, our assays were conducted with a surfeit level of phytase (1,200 U/kg diet), and therefore intestinal phytase activities would be relatively unimportant. Kornegay *et al.* (1996) concluded that phytase-mediated increases in phytate-P utilization were reduced with increasing dietary Pi levels when diets were supplemented with 250, 500, and 750 U/kg phytase. The ability of 1,000 U/kg phytase to improve phytate-P utilization, however, was not affected by increasing levels of Pi. We cannot state with absolute certainty that our level of phytase (1,200 U/kg diet) was not influenced by increased Pi, but it is highly improbable based on the data reported by Kornegay *et al.* (1996).

Our results shown herein indicate that (excess) phytase added to diets based on SBM does not improve the utilization of SBM protein as assessed by growth assay. Moreover, the data cast serious doubt on suggestions that small increases in dietary available P (ie., 0.10%) will decrease protein utilization, although Johnston and Southern (2000b) did observe decreased amino acid

digestibility when 0.10% Pi was added to a P-deficient diet for finishing pigs. Our level of phytase supplementation was generous (1,200 U/kg)...over twice the level being used in practice. Hence, even if excess P were to decrease exogenous or endogenous phytase activity in the gut, the plethoric level of phytase used in our studies herein should have circumvented this effect.

Questions have arisen regarding the effects of Pi levels, if any, on phytate hydrolysis. It has been assumed that phytate binding of protein reduces protein digestibility, and therefore protein utilization. If microbial phytase supplementation causes the release of P from the phytate complex, it is logical to assume that protein should also be released, since the protein that is purportedly bound to phytate is bound to the phosphate moieties of phytate. Ballam *et al.* (1985) concluded that phytate hydrolysis was not severely impaired in chicks fed diets containing 0.80% available P (0.68% P from inorganic sources) compared with those fed the corn-soybean meal basal diet (0.12% available P) without supplemental P. Our amino acid-deficient (12% CP) nursery pig diet, and diets in chick assays 1, 4, and 7 contained nonphytate P levels that were at or slightly above NRC (1998; 1994) nutrient recommendations. Thus, our levels of available P should not have greatly impaired the hydrolysis of phytate. These observations only add veracity to the results obtained in the pig assay and chick assays 1, 4, and 7. Under the conditions of our assays, therefore, not only would the levels of Pi alone not appreciably hinder phytate hydrolysis, but also the surfeit level of supplemental phytase (1,200 U/kg diet) should easily conteract the potential adverse effects, if any, of Pi on phytase activity.

The location of phytase activity within the gastrointestinal tract also is relevant to properly addressing the concerns regarding the potential effects of excess levels of Pi on the potential protein efficacy of phytase. The stomach and small intestine were reported to be the greatest sites of supplemental phytase activity in young pigs fed soybean meal-based diets supplemented with 1,050 U/kg of microbial phytase (Yi and Kornegay, 1996). Moreover, increasing the (deficient) dietary levels of available P from 0.05 to 0.16% did not affect phytase activity in either the stomach or small intestine. More importantly, the phytate-hydrolyzing action of supplemental phytase in the small intestine occurs in the digesta present in the lumen and not at the brush border sites of absorption. In our opinion, the negative effects on P absorption and utilization associated with increasing dietary levels of Pi and Ca are more related to decreased total absorption and absorption efficiency of P (Pointillart *et al.*, 1985;1989) than to reduced phytate hydrolysis or the inhibition of supplemental phytase activity.

Phytase-mediated phytate hydrolysis does not specifically increase the absorption of P, it merely increases the availability of previously phytate-bound P for intestinal absorption and utilization. The effect of excess Pi, therefore, does not affect phytate-P digestibility and P absorption by impairing phytate hydrolysis, but is more related to altered or impaired Ca and P absorption patterns. If phytase truly does have the capacity to improve protein utilization, the addition of phytase to amino acid-deficient diets fed to young chicks and pigs would have increased the digestibility and availability of amino acids for absorption and utilization, regardless of dietary levels of Pi.

Our pig assay, and chick assays 1, 4 , and 7 (diet 2) were conducted using amino acid-deficient levels of protein, and available P levels that were at or slightly above NRC (1994;1998) nutrient

recommendations. Yi *et al.* (1996a) concluded that the greatest efficacy of phytase for consistently improving amino acid digestibility and nitrogen utilization was observed in poults either fed protein-deficient diets containing adequate P, or protein-adequate diets containing deficient levels of available P. Johnston and Southern (2000b), however, found that protein-adequate and P-deficient diets for pigs did not allow positive responses in phytase-mediated amino acid digestibility. Ravindran *et al.* (2000) observed that increasing available P from 0.23% to 0.45% in amino acid-adequate diets fed to broilers reduced P digestibility, or absorption efficiency *per se*, as anticipated, but did not affect ileal N digestibility or retention. The addition of phytase improved N retention, but these effects were independent of dietary phytic acid and available P levels. Increasing the dietary concentration of phytic acid was observed to negatively impact N and amino acid digestibilities, but dietary levels of available P did not influence N digestibility. Although amino acid digestibility in broilers was improved to a greater extent with phytase addition to P-deficient diet compared to P-adequate diets, the addition of phytase (400 and 800 U/kg) still elicited significant improvements in amino acid digestibility for broilers fed P-adequate diets. The observations of Ravindran *et al.* (2000) and Yi *et al.* (1996a), clearly indicate that phytase-mediated improvements in amino acid digestibility are detectable in nonruminant animals fed protein-deficient diets containing nutritionally-adequate (NRC, 1994) levels of available P.

It has been alleged that phytase supplementation to amino acid-deficient diets containing adequate levels of available P would inhibit, and therefore mask the potential phytase-mediated increases in protein utilization. Our data clearly refute this claim. In chick assay 7, growth performance and PER were increased when the P-deficient basal diet was supplemented with either Pi or phytase. Furthermore, supplementing Pi sources to provide marginal excesses of P and Ca did not affect any of the response criteria. The findings of Yi *et al.* (1996a) and Ravindran *et al.* (2000) are consistent with these findings. Thus, the potential improvements in amino acid digestibility mediated by phytase do not consistently transmit into noticeable improvements in economically important growth traits.

The current state of knowledge does not warrant the broad-scale supplementation of microbial phytase to swine and poultry diets as a means of improving protein and amino acid digestibility and reducing nitrogen excretion. Nevertheless, the primary dilemma faced by producers is a consortium of incongruent information. Thus, potential phytase-mediated increases, if any, in protein and amino acid digestibility have rarely manifested in improved growth performance and feed efficiency. In keeping with the best interests of the producer, the use of growth responses, therefore, would appear to be the most acceptable and applicable method for evaluating the efficacy of phytase for improving protein-amino acid utilization. The lack of growth responses to phytase supplementation may be caused by currently unknown interactions and possible decreases in the utilization of other nutrients that result in the absence of a net beneficial effect on protein utilization.

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Table 1. Percentage composition of basal diets (as-fed basis)

Ingredient	*Pig assay*[1]	*Chick assays*		
		1,2,3[2]	*4,5,6*[3]	*7*[4]
Cornstarch	to 100.00	30.08	44.64	to 100.00
Dextrose	---	to 100.00	44.64	29.81
Sucrose	30.00	---	---	---
Soybean oil	4.00	5.00	5.00	5.00
Dicalcium phosphate	1.80	2.08	2.04	1.46
Limestone	1.00	1.30	1.30	1.48
K_2CO_3	---	---	0.90	0.50
$MgSO_4 \bullet 7H_2O$	---	---	0.35	0.35
$NaHCO_3$	---	0.40	---	---
Chick trace mineral mix[5]	---	0.15	0.15	0.15
Pig trace-mineral salt mix[6]	0.35	---	---	---
Vitamin mix	0.30[7]	0.20[8]	0.20[8]	0.20[8]
NaCl	0.41	0.35	0.50	0.50
Choline chloride	---	0.20	0.20	0.20
Antibiotic premix[9]	0.50	---	---	---
$CuSO_4 \bullet 5H_20$	0.08	---	---	---
Bacitracin MD premix[10]	---	0.025	0.025	0.025
DL-α-tocopheryl acetate (20 mg/kg)	-	+	+	+
Ethoxyquin (125 mg/kg)	-	+	+	+

[1] Soybean meal (47.0% CP) was added at the expense of cornstarch to provide either 12 or 24% CP. The basal diet contained 0.74% Ca and 0.33% available P.

[2] Soybean meal (46.6% CP) was added at the expense of cornstarch to provide either 5, 10, or 15% CP. The basal diet contained 0.94% Ca and 0.39% available P.

[3] Soybean meal (46.6% CP), corn gluten meal (62.9% CP), and casein (88.3% CP) were added at the expense of cornstarch:dextrose (2:1, w/w) to provide exactly 10% CP. The basal diet contained 0.94% Ca and 0.39% available P.

[4] Soybean meal (46.6% CP) was added at the expense of cornstarch to provide 18% CP. The basal diet contained 0.95% Ca and 0.35% available P.

[5] Provided the following (per kilogram of diet): Fe, 75 mg ($FeSO_4 \bullet H_2O$); Zn, 75 mg (ZnO); Mn, 75 mg (MnO); Cu, 5 mg ($CuSO_4 \bullet 5H_2O$); I, .75 mg (ethylene diamine dihydroiodide); Se, .1 mg (Na_2SeO_3).

[6] Provided the following (per kilogram of diet): Fe, 90 mg ($FeSO_4 \bullet H_2O$); Zn, 100 mg (ZnO); Mn, 20 mg (MnO); Cu, 8 mg (CuO); I, .35 mg (CaI_2); Se, .3 mg (Na_2SeO_3); NaCl, 3 g.

[7] Pig vitamin mix provided the following (per kilogram of diet): retinyl acetate, 2,273 µg ; cholecalciferol, 16.5 µg; dl-∝-tocopheryl acetate, 88 µg; niacin, 33 µg; d-Ca pantothenate, 24.2 µg; riboflavin, 8.8 µg; vitamin B_{12}, 0.035 µg; menadione sodium bisulfite complex; 4.4 µg; choline chloride, 324 mg.

[8] Chick vitamin mix provided the following (per kilogram of diet): thiamin•HCl, 20 mg; nicotinic acid, 50 mg; riboflavin, 10 mg; D-Ca pantothenate, 30 mg; vitamin B_{12}, 0.04 mg; pyridoxine•HCl, 6 mg; D-biotin, 0.6 mg; folic acid, 4 mg; menadione dimethylpyrimidinol bisulfite, 2 mg; ascorbic acid, 250 mg; cholecalciferol, 15 µg; and retinyl acetate, 1,789 µg.

[9] Provided 110 mg chlortetracycline, 110 mg sulfamethazine, and 55 mg of penicillin per kilogram of complete diet.

[10] Added to provide 27.5 mg/kg bacitracin MD.

Table 2. The effect of microbial phytase on crude protein-amino acid utilization of nursery pigs fed amino acid-deficient or –adequate diets (pig assay)[1]

Dietary CP Level (%)[2]	*Phytase*[3]	*Weight gain, g/d*[4]	*Feed intake, g/d*[4]	*Gain/feed, g/kg*[4]	*PER*[4,5]
12	-	344	798	431	3.60
12	+	320	722	443	3.69
24	-	612	932	657	2.74
24	+	613	928	660	2.76
Pooled SEM		14	27	31	0.10

[1] Data represent means of four pens of four PIC pigs during a 17-d feeding period; average initial weight was 10.6 kg.
[2] Soybean meal (47.0% CP) was added at the expense of cornstarch to provide either 12 or 24% crude protein.
[3] Added at the expense of cornstarch to provide 1,200 U/kg diet.
[4] Protein level main effect ($P < 0.01$).
[5] PER (Protein efficiency ratio) = weight gain (g) per g protein intake.

Table 3. Summary of three separate experiments evaluating slope-ratio phytase efficacy for improving protein utilization in dehulled soybean meal fed to young chickens by at three combinations of excess dietary calcium and available phosphorus (chick assays 1, 2, and 3)[1]

Assay #	*Dietary additions*[2,3]	*Weight gain, g*[4]	*Gain:feed, g/kg*	*Protein intake, g*	*PER*[5]
1. *0.45% available P, 1.00 % Ca*					
	1. 5% CP from SBM	19	101	9.4	2.03
	2. 10% CP from SBM	96	312	30.8	3.12
	3. 15% CP from SBM	208	473	66.0	3.15
	4. As 1 + 1,200 U/kg phytase	30	134	11.2	2.68
	5. As 1 + 1,200 U/kg phytase	98	311	31.5	3.11
	6. As 1 + 1,200 U/kg phytase	197	465	63.5	3.10
	Pooled SEM	5	13	1.2	0.22
2. *0.45% available P, 1.50% Ca*					
	1. 5% CP from SBM	26	112	11.6	2.24
	2. 10% CP from SBM	111	320	34.7	3.20
	3. 15% CP from SBM	208	466	66.9	3.11
	4. As 1 + 1,200 U/kg phytase	32	135	11.9	2.69
	5. As 1 + 1,200 U/kg phytase	125	338	37.0	3.38
	6. As 1 + 1,200 U/kg phytase	203	458	66.5	3.05
	Pooled SEM	8	12	1.6	0.19
3. *0.675% available P, 1.50% Ca*					
	1. 5% CP from SBM	23	101	11.4	2.03
	2. 10% CP from SBM	126	328	38.4	3.28
	3. 15% CP from SBM	224	470	71.5	3.13
	4. As 1 + 1,200 U/kg phytase	26	110	11.8	2.20
	5. As 1 + 1,200 U/kg phytase	119	320	37.2	3.20
	6. As 1 + 1,200 U/kg phytase	211	450	70.3	3.00
	Pooled SEM	6	13	0.8	0.24

[1] Data are mean values of four pens of four female chicks during the period 8 to 21-d posthatching; avg. initial weights in each experiment were for assay 1) 88 g; 2) 94 g; and 3) 100 g.

[2] Soybean meal (46.6% CP) was added at the expense of dextrose to provide 5, 10, or 15% CP.

[3] Calcium and phosphorus supplements to the basal diet were provided as $CaCO_3$ and dicalcium phosphate; supplements were provided to maintain desired levels of available phosphorus and calcium; phytase was provided as Natuphos® phytase.

[4] Multiple linear regression analysis of 13-d weight gain (g) on crude protein intake (g) resulted in the following equation: For experiment 1) $Y = -6.84 + 3.26X_1 + 3.23X_2$, $R^2 = 0.99$; 2) $Y = -4.54 + 3.21X_1 + 3.22X_2$, $R^2 = 0.99$; and 3) $Y = -7.64 + 3.29X_1 + 3.17X_2$, $R^2 = 0.99$ where X_1 = no supplemental phytase, X_2 = 1200 U/kg supplemental phytase. No effect ($P > 0.16$) of phytase supplementation was observed, regardless of dietary calcium and available phosphorus levels.

[5] PER (protein efficiency ratio) = weight gain (g) per g protein intake.

Table 4. Summary of three separate experiments evaluating protein efficiency ratios for chicks fed diets with 10% protein from casein, corn gluten meal, or soybean meal as affected by microbial phytase at three combinations of excess dietary calcium and available phosphorus (chick assays 4, 5, and 6)[1]

			Test ingredient[2]						
			Casein		*Soybean meal*		*Corn gluten meal*		*Pooled*
Assay	*Dietary additions*	*Phytase*[3]	-	+	-	+	-	+	*SEM*[4]
4. *0.45% available P, 1.00 % Ca*[5]									
	Weight gain, g		62	53	108	101	17	17	3
	Protein intake, g		18.5	17.5	27.0	25.7	11.7	11.6	0.5
	PER[6]		3.35	3.03	4.00	3.93	1.45	1.47	0.11
5. *0.45% available P, 1.50 % Ca*[5]									
	Weight gain, g		55	59	110	107	19	16	2
	Protein intake, g		18.6	19.8	27.9	27.9	12.8	12.4	0.5
	PER[6]		2.96	2.98	3.94	3.83	1.48	1.29	0.10
6. *0.675% available P, 1.50 % Ca*[5]									
	Weight gain, g		60	75	107	108	14	16	5
	Protein intake, g		19.8	22.2	28.4	28.5	12.4	12.2	1.0
	PER[6]		3.03	3.38	3.77	3.79	1.13	1.31	0.11

[1] Data are mean values of four pens of four female chicks during the period 8 to 17-d posthatching; avg. initial weights in each experiment were for assay 4) 92 g; 5) 97 g; and 6) 100 g.

[2] Soybean meal (46.6% CP), corn gluten meal (62.9% CP), and casein (88.3% CP) were added to provide exactly 10% CP.

[3] Phytase (Natuphos®) was added to provide 1,200 U/kg diet.

[4] Factorial ANOVA results were the same for all three assays: protein source effect ($P < 0.01$) for weight gain, protein intake, and PER; phytase effect not significant ($P > 0.10$) for all criteria; and protein source x phytase interaction not significant ($P > 0.10$) for all criteria.

[5] Calcium and phosphorus supplements to the basal diet were provided as $CaCO_3$ and dicalcium phosphate; supplements were provided to maintain desired levels of available phosphorus and calcium.

[6] PER (protein efficiency ratio) = weight gain (g) per g protein intake.

Table 5. Protein utilization from dehulled soybean meal fed to chicks at varying levels of calcium and available phosphorus in the absence and presence of phytase (chick assay 7)[1]

Diet[2]	*Weight gain, g*	*Gain/feed, g/kg*	*Protein intake, g*	*PER*[3]	*Tibia ash*	
					%[4]	*mg*
1. Basal SBM (18% CP)[5]	238	538	79.6	2.99	44.4	470
2. As 1 + 0.10% Pi + 0.05% Ca	249	546	82.1	3.03	45.1	489
3. As 1 + 1,200 U/kg phytase	256	557	82.7	3.10	45.0	494
4. As 2 + 0.10% Pi + 0.05% Ca	254	549	83.3	3.05	44.6	484
5. As 2 + 1,200 U/kg phytase	255	551	83.3	3.06	44.9	489
Pooled SEM[6]	6	5	1.5	0.03	0.4	11

[1] Data are mean values of four pens of four female chicks during the period 8 to 20-d posthatching; average initial weight was 97 g.

[2] Calcium and phosphorus supplements to the basal diet were provided as $CaCO_3$ and KH_2PO_4; phytase was provided as Natuphos® phytase.

[3] PER (protein efficiency ratio) = weight gain (g) per g protein intake.

[4] g ash per 100 g dry tibia weight.

[5] The basal diet was analyzed to contain 18% protein, 0.95% calcium, and 0.53% phosphorus (0.35% estimated available P); the diet is deficient in methionine (Emmert *et al.*, 2000) and marginal in calcium and available phosphorus (NRC, 1994).

[6] Factorial single df analysis of treatment differences consisted of deficient P vs adequate P (diet 1 vs diets 2-5), $P < .07$ for gain and $P < 0.05$ for gain/feed and PER; adequate P vs 0.10% excess P (diets 2 and 3 vs diets 4 and 5), not significant ($P > 0.10$) for any criterion; inorganic P vs phytase supplementation (diets 2 and 4 vs diets 3 and 5), not significant for any criterion; interaction of adequate vs excess P x inorganic P vs phytase (within diets 2-5), not significant for any criterion.

The Growth Performance of Two Lines of Pig Reared under Two Differing Environmental Conditions

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Introduction

There have been numerous studies reporting the differences in performance levels and carcass characteristics between contrasting breeds and lines of pigs. Additionally, there is also a body of literature reporting that increased group size and reduced floor space allowance (crowding) results in reduced feed intakes and growth rates (Edmonds et al., 1998; Hyun et al., 1998). It is well known that the animal's environment dictates the level to which it will express its genetic potential. Thus, the concept of genotype × environmental interactions (G × E) becomes important. Merks (1986), defined a G × E as a change in the relative performance of two or more genotypes measured in two or more environments. Several researchers have shown that genotype × environmental interactions may exist in swine populations (Bidanel and Ducos, 1996; Merks, 1989). As commercial producers continue to try new techniques to increase the efficient use of their facilities such as large group sizes and increased stocking densities, the presence of genotype × environmental interactions may become more important. The objective of this study was to investigate the interaction between genetic growth potential and rearing environment.

Materials and Methods

Experimental Design and Treatments. This study compared three treatments with three replicates over time. The treatments were: sire line (line A vs line B, [Pig Improvement Company, U.S.A., Franklin, KY]), environment (spacious vs crowded), and sex (barrows vs gilts). The protocol for this study was approved by the University of Illinois Laboratory Animal Care Committee.

Animals and Management A total of 736 pigs were put on test at a average of approximately 40 kg live weight. Line A was Pietrain-based and line B was a synthetic containing Large White, Landrace, Duroc and Pietrain. These two lines were chosen on the basis of previous data to represent the range in growth rate among sire lines from this genetic source (Miller et al., 2000). Sires from line A (n = 8) and line B (n = 9) were mated to PIC Camborough 22 dams. All lines used in this study had been tested as free of the detrimental alleles of both the Halothane and RN genes. Pigs were housed in like-genotype, like-sex groups and were allocated to test group on the basis of sire and weight.

The study was conducted in a mechanically ventilated building at the University of Illinois Swine Research Center which had part-solid, part-slotted floors. The spacious environment consisted of small groups (4 pigs) with a more than adequate floor space allowance (0.93 m^2/pig for the entire grow-finish period). Pigs in the crowded environment were in larger groups (12 pigs) with a

reduced floor space allowance [(0.37 and 0.56 m^2/pig for the grower (40 to 80 kg) and finisher (80 to 120 kg) phases, respectively)]. At a mean pen weight of 80 kg, the crowded environment pens were enlarged by widening the existing pen, keeping a constant ratio of solid to slotted floor. Pigs were given ad libitum access to feed from a two-hole feeder and were fed on a three-phase dietary program; diets were based on corn and soybean meal (Table 1).

Measurements. Pigs were individually weighed every 14 d in the first and third replicate and every 21 d in the second replicate. In addition, animals were weighed weekly when they approached 80 kg, for pen size adjustment for the crowded environment, and 120 kg, the end of test weight. The coefficient of variation for each pen was calculated at the start and the end of the test. Feed additions were recorded and feeder weights were taken at each weighing. Ultrasound scanning was performed on every pig at each weigh date. Fat-free lean percentage was calculated at 120 kg live-weight using an equation presented by Cisneros et al. (1996).

Results and Discussion

Effect of Sire line. There were no significant ($P > 0.05$) interactions between genotype and environment fro any of the traits. No difference ($P > 0.05$) in the coefficient of variation of end weights was found between line A and line B pigs (Table 2). Line A animals grew significantly slower ($P < 0.05$) during both phase 2 (80 to 120 kg) and the overall test period, and required more days to reach slaughter weight compared to line B pigs (Table 3). Line B pigs had higher daily feed intake ($P < 0.05$) than line A for phase 2 but not for phase 1 or the overall period, however, gain:feed was lower for line A pigs for phase 1 and for the entire test period (Table 3). Line A was a Pietrain-based line and studies conducted with lines based on this breed have generally shown slower growth and poorer feed efficiency for this genotype (Howard and Smith, 1977). In addition, line A pigs had greater loin depths ($P < 0.05$), and similar fat depth and fat-free lean percentage when compared to line B pigs (Table 3).

Effect of Environment. Environment did not have any effect on the coefficient of variation of live weight at the end of the study (Table 2). Pigs placed in the crowded environment had lower ($P < 0.05$) average daily gain and average daily feed intake during phases 1 and 2 and the entire test period compared to pigs reared in the spacious environment. Gain:feed was also lower ($P < 0.05$) for the crowded environment pigs during phase 1 and the overall test period, but did not differ for phase 2 (Table 3). Edmonds et al. (1998) reported similar results for animals placed in crowded pens. However, most studies have shown that a reduction in floor space allowance results in a reduction in average daily gain and average daily feed intake with no effect on gain:feed (NCR-89, 1993; McGlone and Newby, 1994; Brumm and Miller, 1996).

Effect of Sex. Gilts had less ($P < 0.05$) variation in live weight at the end of the study than barrows (Table 2). Barrows grew faster ($P < 0.05$) during phase 2 and the entire test period and consumed more feed ($P < 0.05$) and had poorer feed efficiency ($P < 0.05$) compared to gilts throughout the trial (Table 3). Gilts had less ($P<.05$) backfat and a greater loin eye depth and a higher fat-free lean percentage compared to barrows (Table 3). Other studies have reported higher average daily gain and average daily feed intake, lower gain:feed, greater backfat

thickness with less loin eye area for barrows compared to gilts (Cisneros et al., 1996; Ellis et al., 1996).

Conclusions

1. The differences in growth rate and carcass characteristics between the sire lines used in this study emphasizes the importance of selecting a sire line that meets the goals of the specific swine operation.

2. The large reduction in growth rate for the pigs reared in the crowded environment highlights the impact that group size and floor space allowance has on growth performance.

3. This study reduces the concern of negative effects on carcass lean content for pigs that are reared in a crowded environment with a lower than recommended floor space allowance.

4. The absence of genotype × environmental interactions in the present study indicates that the two sire lines responded similarly to the two environments, however, more studies need to be conducted with more genetic lines and environments to confirm these results.

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Table 1. Diet formulations and percentage composition[a]

	Live weight range, kg		
Ingredient (%)	40-70	70-100	100-120
Corn	70.120	74.690	80.491
Soy bean meal (48%)	25.050	20.700	15.154
Soybean oil	1.500	1.500	1.500
L-Lysine-HCL	0.120	0.085	0.195
DL-Methionine	0.030	0.015	0.000
Dicalcium phoshphate	1.570	1.390	1.220
Ground Limestone	0.760	0.780	0.790
Trace mineral salt[b]	0.350	0.350	0.350
Vitamin mix[c]	0.200	0.200	0.200
Copper sulfate	0.100	0.100	0.100
Tylan (2%)	0.200	0.200	0.200
Calculated compostion			
Crude protein, %	18.200	16.500	14.300
Lysine, %	1.050	0.900	0.820
Calcium, %	0.750	0.700	0.615
Phosphorous, %	0.650	0.600	0.525
ME, kcal/kg	3384	3390	3386

[a] As-fed basis.

[b] Each kilogram of mix contained the following: Se, 85.7 mg; I, 100 mg; Cu, 2.3 g; Mn, 5.7 g; Fe, 25.7 g; Zn, 28.6g; NaCl, 855 g.

[c] Each kilogram of mix contained the following: vitamin A, 3,000,000 IU; vitamin D_3, 330,000 IU; vitamin E, 44,000 IU; vitamin K, 2.2 g; vitamin B_{12}, 17.9 mg; riboflavin, 4.4 mg; d-pantothenic acid, 12.1 g; niacin, 16.5 g; choline chloride, 165 g; and roughage products to 1 kg.

Table 2. Least square means for genetic line, environment and sex for live weights, mortality and removal rate.

Variable	Genotype			Environment			Sex		
	Line A	Line B	Ave SE	Spacious	Crowded	Ave SE	Barrows	Gilts	Ave SE
Number of pens	45	47		46	46		47	45	
Start weight, kg	40.7	41.0	0.28	41.2	40.5	0.28	41.1	40.6	0.28
Switch weight, kg	80.1	80.0	0.63	80.0	80.1	0.63	80.2	79.9	0.63
End weight, kg	120.0	120.7	0.53	120.1	120.6	0.53	120.4	120.3	0.53
Start CV[a], %	7.6	7.5	0.39	7.3	7.8	0.39	8.0	7.1	0.39
End CV[a], %	7.7	7.0	0.50	6.8	7.9	0.50	8.2 [c]	6.5[b]	0.50
No. animals at start	356	380		184	552		380	356	
No. animals died	4	6		1	9		4	6	
No. animals removed	7	12		3	16		14	6	
Percentage of animals dying or removed	3.1	4.7		2.2	4.5		4.7	3.3	

[a] CV = Coefficient of variation was determined using pen means.
[bc] Means in the same row with differing superscripts differ, P≤05.

Table 3. Least square means for genetic line, environment and sex for growth performance.

Variable	Genotype			Environment			Sex			G × E
	Line A	Line B	Ave	Spacious	Crowded	Ave SE	Barrows	Gilts	Ave SE	P value
Days on test	86.1[b]	82.0[a]	0.94	79.5[a]	88.6[b]	0.96	82.6[a]	85.4[b]	93.1	0.59
Average daily gain, g										
Phase 1 (40 - 80 kg)	918	940	10.6	990[b]	868[a]	10.7	942	915	10.6	0.69
Phase 2 (80 - 120 kg)	912[a]	991[b]	14.5	997[b]	906[a]	14.8	979[b]	923[a]	14.5	0.97
Overall (40 - 120 kg)	915[a]	965[b]	9.4	993[b]	887[a]	9.6	961[b]	919[a]	9.4	0.63
Average daily feed intake, g										
Phase 1 (40 - 80 kg)	2350	2320	24.1	2441[b]	2229[a]	24.6	2411[b]	2259[a]	24.1	0.78
Phase 2 (80 - 120 kg)	2905[a]	3082[b]	40.0	3167[b]	2820[a]	40.8	3156[b]	2831[a]	40.1	0.45
Overall (40 - 120 kg)	2627	2701	26.9	2804[b]	2524[a]	27.4	2784[b]	2545[a]	26.9	0.49
Gain : Feed										
Phase 1 (40 - 80 kg)	0.39[a]	0.41[b]	0.004	0.41[b]	0.39[a]	0.004	0.39[a]	0.41[b]	0.004	0.40
Phase 2 (80 - 120 kg)	0.31	0.32	0.003	0.32	0.32	0.003	0.31[a]	0.33[b]	0.003	0.11
Overall (40 - 120 kg)	0.35[a]	0.36[b]	0.003	0.36	0.35	0.003	0.35[a]	0.37[b]	0.003	0.08
Carcass measurements[c], mm										
Fat depth	14.7	14.3	0.29	14.4	14.6	0.29	15.7[b]	13.3[a]	0.29	0.14
Loin eye depth	60.4[b]	56.9[a]	0.38	58.1	59.1	0.38	58.0[a]	59.2[b]	0.38	0.47
Predicted fat-free lean percent[d]	57.1	57.0	0.12	57.0	57.1	0.12	56.6[a]	57.5[b]	0.12	0.07

[ab] Means in the same row with differing superscripts differ, P≤05.

[c] Measured at 120 kg live weight using ultrasound.

[d] Predicted form fate depth and longisimuss measurements using the equation of Cisneros et al., 1996.

The Carcass and Meat Quality Characteristics of Two Lines of Pig Reared under Two Differing Environmental Conditions

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Introduction

There has been limited research carried out on the effects of environmental factors such as group size and crowding on carcass and meat quality characteristics in pigs. A number of studies investigating the effects of rearing environment on carcass and meat quality traits have compared outdoor versus indoor rearing systems and have shown variable results. Pigs reared outdoors have been reported to have darker meat color and no difference in ultimate pH when compared to confinement reared pigs (Wariss et al., 1983). Enfalt et al. (1996) showed that pigs reared in confinement had higher ultimate pH and marbling and lower drip loss than pigs reared outdoors. Other studies have shown no effect of rearing environment on these traits (van der Wal et al., 1993).

The animal's rearing environment will dictate the level to which it will express its genetic potential. An important consideration in choice of the genotype to use in any particular situation is the potential for genotype x environment interactions for the important traits. Commercially, genotype × environment interactions are important because they will dictate the optimum genetic line for use in a particular environment. Several studies have shown that genotype × rearing environment interactions occur in swine populations (Bidanel and Ducos, 1996; Merks, 1989). However, most studies have focused on growth performance and the objective of this study was to investigate the interaction between sire line and environment for carcass and meat quality characteristics in pigs.

Materials and Methods

Experimental Design and Treatments. This study compared three treatments with two replicates over time. The treatments were: sire line (line A vs line B, [Pig Improvement Company, U.S.A., Franklin, KY]), environment (spacious vs crowded), and sex (barrows vs gilts). The protocol for this study was approved by the University of Illinois Laboratory Animal Care Committee.

Animals and Management Line A was Pietrain-based and line B was a synthetic containing Large White, Landrace, Duroc and Pietrain. Sires from line A (n = 7) and line B (n = 8) were mated to PIC Camborough 22 dams. All lines used in this study had been tested as free of the detrimental alleles of both the Halothane and RN genes.

Two replicates of 242 pigs each were put on test at an average of approximately 40 kg live weight. The study was conducted in a mechanically ventilated building at the University of Illinois Swine Research Center which had part-solid, part-slotted floors. The spacious

environment consisted of small groups (4 pigs) with a more than adequate floor space allowance (0.93 m^2/pig for the entire grow-finish period). Pigs in the crowded environment were in larger groups (12 pigs) with a reduced floor space allowance [(0.37 and 0.56 m^2/pig for the grower (40 to 80 kg) and finisher (80 to 120 kg) phases, respectively)]. At a mean pen weight of 80 kg, the crowded environment pens were enlarged by widening the existing pen, keeping a constant ratio of solid to slotted floor. Pigs were given ad libitum access to feed from a two-hole feeder and were fed on a three-phase dietary program; diets were based on corn and soybean meal.

When the mean pen weight reached 120 kg, two individuals were selected at random from each pen to be slaughtered for carcass and meat quality evaluation. Pigs remained in their test groups until they were transported from the farm to the Meat Science Laboratory at the University of Illinois on the afternoon prior to slaughter, where they were held overnight for approximately 16 h prior to slaughter. Animals were mixed with those from other groups during transport and in the lairage where they were held without food but with access to water.

Slaughter and Carcass Evaluation. Pigs (n = 128) were weighed immediately before slaughter. The carcass was split down the midline, the head was removed, and hot carcass weight was recorded. Carcasses were placed in a chiller (4°C) approximately 1-h postmortem where they were held overnight. At 24-h postmortem, cold carcass weight was recorded, and carcass measurements were obtained from the left side of each carcass.

Meat Quality Measurements. At 45-min post mortem, muscle pH was measured on a longissimus sample (approximately 3 g) taken at the level of the 10^{th} rib. At 24-h post mortem, subjective scores for longissimus color, firmness, and marbling were taken on the cut surface of the longissimus at the tenth rib using the procedures described by NPPC (1991) based on five point scales (1 = pale, soft, and devoid of marbling; 5 = dark, firm, and moderately abundant or greater marbling). Minolta color (L*, a*, and b* values) was measured on the cut surface of the loin section at the 10^{th} rib. A loin chops (2.5 cm thick) were cut from the longissimus immediately posterior to the 10^{th} rib. One chop was used to determine drip loss and the other for ultimate pH. A 10-cm section of the longissimus was taken immediately anterior to the last rib for shear force and cooking loss evaluation. The section was vacuum packaged, aged for 7 d at 4°C, then frozen (-20°C).

Shear Force and Cooking Loss. A chop was removed from the frozen loin section for Warner-Bratzler shear force determination thawed for 24 h at 4°C, and cooked to an internal temperature of 70°C. The chops were weighed before and after cooking to determine cooking loss. Shear force was measured on three cores from each chop.

Curing of Hams. The trimmed boneless ham from the left side of half of the carcasses chosen at random from the first replicate (n=64) was frozen for subsequent curing yield evaluation. After all hams were collected they were thawed at 4°C for 72 h and green weights were recorded. Hams were injected to 125% of their weight with a commercial cure solution to result in a finished product that conatained sodium chloride, 2.0%; sodium tripolyphosphate, 0.5%; sodium

erythorbate, 550 ppm; sodium nitrite, 150 ppm, and a pumped weight was recorded. The hams were tumbled at 25 mm Hg vacuum for 4 h, weighed, placed into fibrous casing, then cooked and smoked in a smoke house. Hams were cooled in a chiller at 4°C for 12 h, the cooked chilled weights were recorded, and curing yields were calculated.

Results and Discussion

Effect of Sire Line. There was no difference between the lines for fat depths (Table 1); however, line A progeny had greater ($P < 0.05$) loin eye depth and loin eye area than line B. These findings are similar to those of others who have compared purebred Pietrain or lines base on the Pietrain to other breeds or lines (Howard and Smith, 1977). Line B had higher ($P < 0.05$) 45 min and 24 h pH ($P < 0.05$) values than line A (Table 2). Furthermore, line B was superior for a number of pork quality characteristics measured on the longissimus such as subjective color, firmness, and marbling, and Minolta L* and b* (Table 2). A number of other studies have shown inferior pork quality attributes for Pietrains compared to other breeds (Howard and Smith, 1977; Oliver et al., 1993). A genotype × environment interaction was observed for drip loss ($P < 0.05$, Table 2); line B pigs had a lower drip loss (2.23% units) in the crowded environment than the spacious environment, whereas line A animals showed no difference in drip loss between the two environments (Table 2). However, there was no significant ($P > 0.10$) interaction between genotype, and rearing environment for other indices of PSE such as 45 min pH, color, firmness and Minolta L* values (Table 5). Sire line had no effect on ham pumping characteristics (Table 3).

Effect of Rearing Environment. There were no differences ($P > 0.05$) in fat or muscle measurements taken on the carcass between pigs reared in the two environments (Table 1). Other studies have also reported no difference in fat depths for pigs reared at different floor-space allowances (Brumm and NCR-89, 1996; Edmonds et al., 1998). In the present study, pigs that were housed in the crowded environment did have lower ($P < 0.05$) Minolta L* values, indicating darker muscle color (Table 2); however, there was no difference between the rearing environments for other pork quality traits. Furthermore, there was no difference ($P > 0.05$) in ham pumping characteristics between animals reared in the spacious and crowded environments (Table 3). Enfalt et al. (1996) found a lower ultimate pH, higher drip loss, increased shear force values and reduced intramuscular fat for outdoor compared to indoor reared pigs. However, Jones et al. (1994) and van der Wal et al. (1993) compared pigs from outdoor and indoor production systems and found no differences in longissimus L* scores.

Effect of Sex. Gilts had heavier hot carcass weights, higher dressing percentages and a lower fat depth at the10th rib and a larger loin eye area compared to barrows (Table 1), results that are similar to other studies at this center (Ellis et al., 1996; Cisneros et al., 1996). Subjective color, firmness, and marbling scores on the longissimus were higher ($P < 0.05$) for barrows, indicating that they had darker, firmer muscle with more visible marbling compared to gilts (Table 2). There were no differences between the sexes for any other pork quality traits. Gilts had heavier ($P < 0.05$) green ham weights than barrows; however, no other differences between the sexes were observed for ham pumping characteristics (Table 3). Leach et al. (1996) also reported that

gilts had heavier trimmed boneless ham weights when compared to barrows. Other studies have generally found little difference in muscle quality between barrows and gilts (Barton-Gade, 1987, Ellis et al., 1996).

Conclusions

1. The substantial differences among the sire lines and the limited impact of rearing environment observed in this study suggest that producers should be more concerned about choice of genetic line than about group size and floor space allowance in terms of carcass and meat quality.

2. With the exception of the genotype × environment interaction for drip loss it would appear that there is a limited number of genotype × environment interactions for carcass and pork quality traits which reduces the concern about choosing genotypes for specific environments in these respects.

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Table 1. Least square means for the effects of sire line, environment and sex on slaughter and carcass measurements.

	Sire line			Environment			Sex			G × E[f]
Variable	Line A	Line B	Av. SE	Spacious	Crowded	Av. SE	Barrows	Gilts	Av. SE	P value
Slaughter weight, kg	115.4[a]	118.0[b]	0.76	117.0	116.5	0.77	116.7	116.8	0.78	0.74
Hot carcass weight, kg[e]	-	-	0.39	-	-	-	90.6[a]	91.5[b]	0.33	0.02
Spacious Env.	91.3[d]	89.8[c]								
Crowded Env.	91.7[d]	91.4[d]								
Dressing percentage[e]	-	-		-	-	-	77.6[a]	78.4[b]	0.29	0.03
Spacious Env.	78.2[d]	77.0[c]	0.34							
Crowded Env.	78.6[d]	78.3[d]								
Carcass length, cm[e]	82.5	82.8	0.43	82.7	82.6	0.43	82.7	82.6	0.42	0.11
Tenth rib	20.2	21.7	0.72	20.8	21.0	0.70	22.5[b]	19.3[a]	0.70	0.98
Loin eye depth, cm[e]	6.87[b]	6.66[a]	0.072	6.68	6.86	0.070	6.60[a]	6.93[b]	0.071	0.87
Loin eye area, cm^{2e}	47.9[b]	44.9[a]	0.74	45.4	47.4	0.73	44.2[a]	48.6[b]	0.73	0.30

[ab] Means in the same row with differing superscripts differ, P≤ 0.05.
[cd] Interaction means with differing superscripts differ, P≤ 0.05.
[e] Measurements corrected to a slaughter weight of 116.7 kg using covariance analysis.
[f] Genotype by environment interaction.

Table 2. Least square means for the effects of sire line, environment and sex on pork quality characteristics measured on the longissimus.

	Sire line			Environment			Sex			G × E[f]
Variable	Line A	Line B	Av. SE	Spacious	Crowded	Av. SE	Barrows	Gilts	Av. SE	P value
45 min. pH	6.19[a]	6.37[b]	0.062	6.29	6.27	0.062	6.19	6.37	0.062	0.22
24 hr. pH	5.43[a]	5.48[b]	0.018	5.45	5.45	0.017	5.46	5.44	0.017	0.22
Color[e]	2.0[a]	2.5[b]	0.11	2.1	2.4	0.11	2.5[b]	2.0[a]	0.11	0.31
Firmness [e]	2.0[a]	2.4[b]	0.12	2.2	2.2	0.12	2.4[b]	2.0[a]	0.11	0.98
Marbling[e]	1.9[a]	2.3[b]	0.13	2.1	2.2	0.12	2.5[b]	1.7[a]	0.12	0.83
Minolta L*	49.37[b]	47.42[a]	0.647	49.61[b]	47.17[a]	0.643	48.10	48.69	0.627	0.39
Minolta a*	6.04	5.76	0.725	5.56	6.24	0.726	5.95	5.85	0.720	0.34
Minolta b*	8.36[b]	7.54[a]	0.378	8.09	7.81	0.381	7.92	7.99	0.373	0.51
Drip loss, %							5.87	6.83	0.434	0.04
Spacious env.	6.96[d]	6.69[d]	0.616							
Crowded env.	7.29[d]	4.46[c]								
Cooking loss, %	23.22	23.04	0.669	23.26	23.00	0.666	23.04	23.22	0.647	0.08
Warner Bratzler shear, kg	4.76	4.96	0.234	4.77	4.96	0.242	4.80	4.93	0.234	0 .89

[ab] Means in the same row with differing superscripts differ, P≤ 0.05.
[cd] Interaction means with differing superscripts differ, P≤ 0.05.
[e] Subjective color, firmness, and marbling scores where 1 =pale, soft and devoid of marbling and 5=dark, firm and moderately abundant marbling.
[f] Genotype by environment interaction.

Table 3. Least square means for the effects of sire line, environment, and sex on ham curing characteristics.

	Sire line			Environment			Sex			G × E[i]
Variable	Line A	Line B	Av. SE	Spacious	Crowded	Av. SE	Barrows	Gilts	Av. SE	P value
Green weight, kg	-	-		10.5	10.6	0.38	10.1[a]	11.0[b]	0.38	0.02
Spacious env.	11.0[d]	9.9[c]	0.43							
Crowded env.	10.5[gc]	10.8[d]								
Pumped weight[e], kg	13.3	13.2	0.06	13.2	13.2	0.06	13.2	13.2	0.06	0.70
Pump percentage [ef]	25.2[b]	24.0[a]	0.54	24.4	24.8	0.54	24.5	24.6	0.54	0.89
Cooked weight[eh], kg	11.8	11.9	0.02	11.9	11.9	0.02	11.9	11.9	0.02	0.83
Yield[egh], %	111.5	111.6	0.16	111.6	111.5	0.16	111.5	111.7	0.16	0.34

[ab] Means within a row with differing superscripts differ significantly, $P < 0.05$.
[cd] Interaction means with differing superscripts differ, $P \leq 0.05$.
[e] All measurements corrected to a green ham weight of 10.6 kg using covariance analysis.
[f] Pump percentage = ((pumped weight - green weight)/green weight) x 100.
[g] Yield = (cooked weight/green weight) x 100.
[h] Cooked weight and yield are corrected to a pump percentage of 24.6 using covariance analysis.
[i] Genotype by environment interaction.

Signature-Tagged Mutagenesis to Identify Virulence Genes in *Salmonella choleraesuis*

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Abstract

Signature-tagged mutagenesis is a functional genomics approach to identify bacterial virulence genes by simultaneously screening multiple mutants in a single host animal. Avirulent (attenuated) mutants are identified by negative selection (failure to colonize the host). The method was recently developed to investigate *Salmonella typhimurium* in a mouse model of human typhoid fever. We modified the protocol to investigate virulence genes of *S. choleraesuis* in its natural host, the pig. First, we generated random, knock-out (null) mutations in *S. choleraesuis* using transposon-mediated insertion of unique, signature-tagged (40 bp), kanamycin resistance cassettes. To validate the modified protocol, a test pool of 45 mutants was inoculated orally or intraperitoneally (systemic infection) into pigs. Three of the mutants were not identified from cultures of the mesenteric lymph nodes obtained four days after infection. Attenuation of these three candidate attenuated mutants was confirmed by mixed challenge growth experiments (growth of a 1:1 mixture of mutant and wild type *S. choleraesuis*) in broth cultures and in pigs. All three mutants were attenuated for infection of pigs as the mutants were at a marked disadvantage relative to the wild type bacteria for growth. For one mutant, the competitive indices (ratios of mutant to wild type bacteria) were 0.15 and 0.03 for bacteria in the intestines and mesenteric lymph nodes, respectively; for the other two mutants, the indices were about 0.001 in either tissue. In contrast, none of the mutants were at a growth disadvantage in broth cultures. As an additional control, two random mutants recovered from the pigs inoculated with the pool (and therefore not candidates for attenuation) were also tested in the mixed challenge experiments. As expected, the competitive growths of these mutants in culture medium and in pigs were similar to that of the wild type. In one of the three attenuated mutants the inactivated gene has been identified, after cloning and sequencing, as *hilA* . In mouse models of *S. typhimurium* infection, *hilA* is associated with enteric invasion, but not systemic proliferation. Because the *hilA* mutant failed to colonize pigs irrespective of oral or systemic inoculation, its function in pigs is likely to be more complex than in the mouse. Our data show signature-tagged mutagenesis can be used to identify *Salmonella* genes associated with virulence in pigs. As we continue screening mutants, we expect to identify novel genes related to pathogenicity and we are expanding our studies to include *S. typhimurium*.

Introduction

Salmonella choleraesuis causes over 95% of all swine salmonelloses and, although host-adapted to pigs, occasionally causes a severe form of foodborne salmonellosis in humans (Schwartz, 1999). The reservoir for *S. choleraesuis* is the pig intestine. Disease typically involves systemic invasion. Little is known about the mechanisms (factors) involved in disease pathogenesis (Gray & Fedorka-Cray, 1996). Bacterial pathogens typically have multiple virulence factors; for example, at least 60 genes have a role in *S. typhimurium* infections of mice (Groisman & Ochman, 1997). Sequencing the genome of a pathogen will predict relatively few and confirm none of the virulence factors. An efficient, direct method to investigate virulence factors is to use functional genomics. A functional genomics method was recently developed using signature-tagged transposon mutagenesis to investigate *S. typhimurium* in mice as a model of human typhoid fever (Hensel et al., 1995; Holden & Hensel, 1998; Perry, 1999). Virulence genes of bacteria that infect multiple hosts species are not necessarily the same among different potential hosts (Tsolis et al., 1999). We have adapted the

signature tag protocol to scan *S. choleraesuis* for virulence genes important for infecting its natural host, the pig.

Goal

To adapt the signature-tagged mutagenesis protocol to identify genes of *Salmonella choleraesuis* important for virulence (pathogenicity) in pigs.

Material and Methods

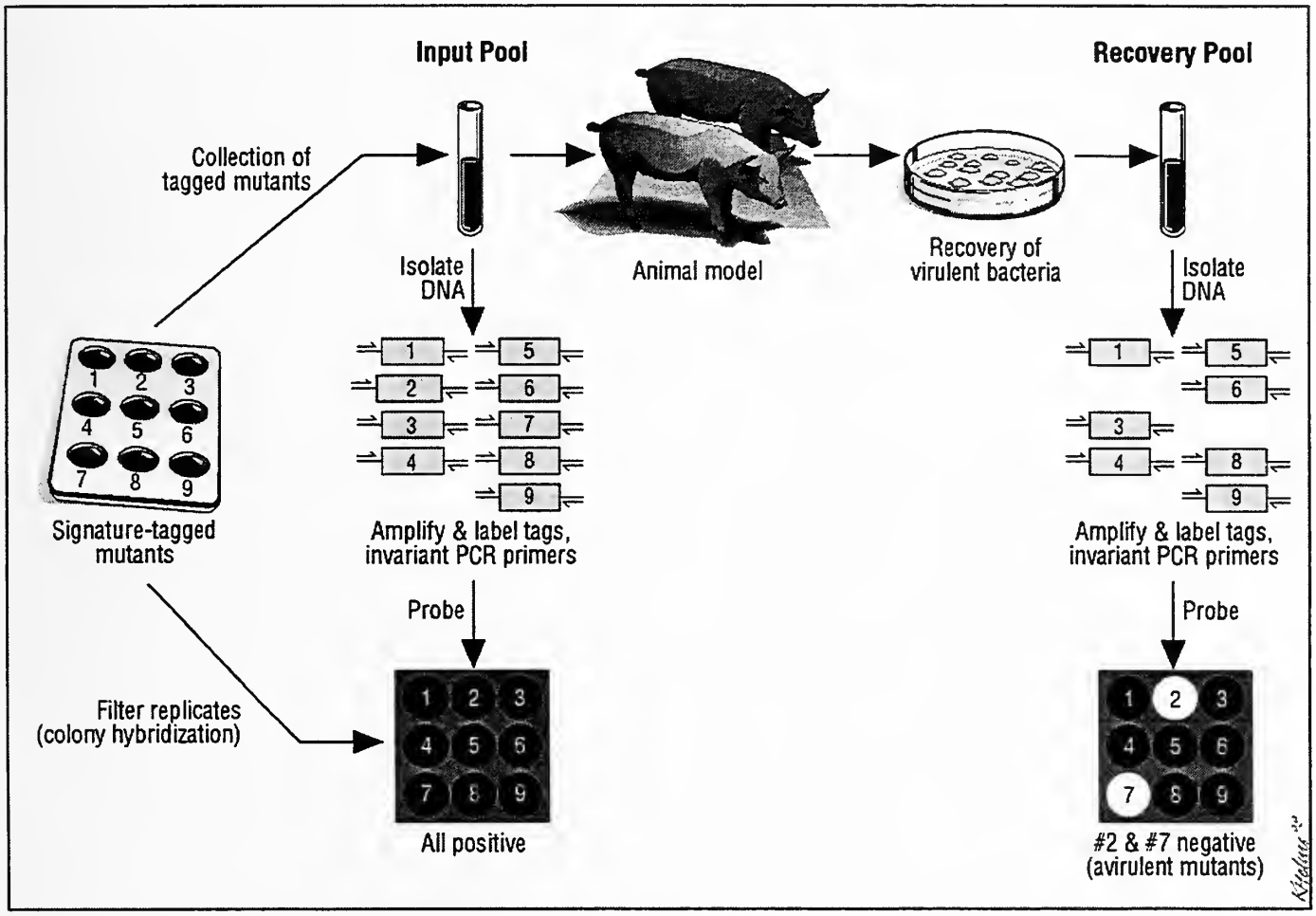

Figure 1. Signature-tagged mutagenesis screening protocol for *Salmonella* pathogenicity genes in swine. The protocol is a negative selection method to scan the bacterial genome for genes which, when inactivated, cause bacteria to loose their ability to colonize host pigs. Mutants have a random gene inactivated by the insertion of a signature-tagged cassette. The signature tags are unique 40 bp double-stranded oliogonucleotides. The Recovery pool (*Salmonella* isolated from inoculated pigs) is compared to the Input pool (inoculum) by colony hybridization probing with labeled signature tags generated by PCR amplification. If the mutant is in the Input pool but not the Recovery pool, the gene tagged in that mutant is a candidate for having a role in pathogenesis. The half arrowheads indicate invariant PCR primers; the primer sequence is excised from the labeled PCR amplicon before probing.

Our protocol to identify virulence genes of *S. choleraesuis* by signature-tagged mutagenesis is diagramed in Figure 1. The protocol is a negative selection method to scan the bacterial genome for genes which, when inactivated, cause bacteria to loose their ability to colonize the host. Mutants have a random gene inactivated by transposon-mediated insertion of a signature-tagged cassette. The signature tags are from a pool of 2×10^{17} double-stranded 40 bp oligonucleotides. If an Input pool mutant is not recognized by the probe from the Recovery pool, the gene tagged and inactivated in that mutant is expected to have a role in pathogenicity.

In our study, we generated signature-tagged mutants of *S. choleraesuis* using Holden's protocol (Holden & Hensel, 1998). The mutants were generated from a nalidixic acid resistant colony we derived from a virulent isolate of *S. choleraesuis* (isolate #537) supplied by Dr. Minion of Iowa State University. We established that doses of 10^7 to 10^8 colony forming units (cfu) induced infection in growing pigs without causing moribund pigs within four days. For protocol validation, we inoculated four 7-week-old pigs with 10^8 cfu of a pool of 45 signature-tagged mutants of *S. choleraesuis*. Two pigs were inoculated orally and two pigs were inoculated intraperitoneally. Four days after inoculation, the pigs were euthanized and *S. choleraesuis* was isolated from the mesenteric lymph nodes of each infected pig using brilliant green agar supplemented with nalidixic acid. Total genomic DNA (chromosomal and large plasmid) was isolated from the Input pool (inoculum) and the pools of *S. choleraesuis* recovered from the inoculated pigs. The bacterial DNA from the two pigs inoculated orally were combined, whereas the DNA from the pigs inoculated intraperitoneally were left as separate samples for analysis of signature tags. Using the DNA from the three Recovery pools, radioactive probes complementary to the signature tags were synthesized by PCR. The probes were hybridized to colony blots of the inoculum to identify those mutants that were recovered from the inoculated pigs. DNA of the Input pool was run in parallel as a control.

Mutants not identified in the pool of bacteria recovered from inoculated pigs (Recovery pool) were candidates for being attenuated (having a virulence gene tagged and inactivated). Attenuation was confirmed using competitive indices after mixed challenge growth (Darwin & Miller, 1999). These indices were the ratios of mutant to wild type bacteria following expansion in mixed cultures (in vitro index) or following mixed challenge infection of pigs (in vivo index). Indices were determined for the three candidate attenuated mutants (those not in Recovery pool) and, for a control, two random non candidates (mutants in the Recovery pool). For the in vitro competitive growth, 1 ml LB broth of the mutant and of wild type *S. choleraesuis* (each approximately 10^5 cfu/ml) were mixed and incubated until expanded more than 10,000-fold.

For the in vivo competitive growth, two pigs for each mutant were inoculated orally with 10^7 cfu of *S. choleraesuis*; the inocula were a 1:1 mixture of mutant and wild type bacteria. Three days following inoculation, the pigs were euthanized and *S. choleraesuis* was cultured from the mesenteric lymph nodes and from pooled intestinal contents and mucosa at the ileocecal junction. The cultures were done on selective brilliant green agar supplemented with nalidixic acid. In addition, the agar used for the intestinal cultures contained streptomycin and sulfadizine to eliminate growth of the enteric flora. After mixed expansion, either in vitro or in vivo, the phenotype of approximately 1,000 colonies was determined by replica printing the colonies to LB agar supplemented with kanamycin. (The mutants are kanamycin resistant but the wild type is sensitive and thus does not grow on the replicas.) If over 1,000 colonies were phenotyped as wild type without identifying a single mutant, the index was listed as less than 1/total number of colonies phenotyped. The in vivo competitive indices were averaged for the two pigs. The competitive index for each mutant was normalized for any differences in input ratios. The cfu in the input (the starting cultures or pig inocula) were estimated based on optical density at 600 nm and then measured by colony counts after overnight incubation on agar. The equation for the competitive index was

(Mo/Wo)/(Mi/Wi) where **M** is mutant, **W** is wild type, **o** is cfu in output, and **i** is cfu in input. An index of 1 indicates no advantage to either the mutant or wild type isolate; mutants with an index of < 0.4 were considered attenuated (Darwin & Miller, 1999).

The first step in characterizing the candidate attenuated genes was to clone and sequence a segment of the tagged gene. Southern blot analyses were done with a ^{32}P labeled kanamycin gene probe to identify restriction enzymes that excise the kanamycin cassette with a large (3 to 5 kb) segment of the adjacent genome of the mutant. *Kpn*I, *Eco*RI, or both generated large fragments in the three mutants and were used for cloning. The fragments were cloned by ligating the digested DNA from mutants into pUC18, transforming the ligated plasmid into *E. coli* DH5α, and selecting for ampicillin resistance (pUC18 encoded) and kanamycin resistance (encoded in kanamycin, signature tag cassette of the mutant). Genetic Engineering Center of the University of Illinois sequenced about 500 bp of the 5' end of the cloned *KpnI* segment of the first mutant. The sequencing primer was complementary to the 3' end of the kanamycin cassette (*Kpn*I incision site is in the 5' end of the cassette) (Holden & Hensel, 1998).

Results

Following oral inoculation of pigs with the pool of 45 signature tagged mutants, over 10,000 cfu of *S. choleraesuis* were isolated from the mesenteric lymph nodes of each of the pigs inoculated orally (two pigs) or intraperitonelly (two pigs). The amplified labeled signature tags from these Recovery pools failed to hybridize with two mutants (A5 and F1) in colony blot hybridization assays of the inoculum (Figure 2, mutants with large circles; Input pool [Blot A] and Recovery pools [Blots B, C, and D]). Therefore, apparently these two mutants were unable to establish infection in the pigs and were candidate attenuated mutants. The probe for a third mutant (Figure 2, mutant B2 with small circle) failed to hybridize in one of three preparations (Figure 2, Blot C), identifying a third potential attenuated mutant.

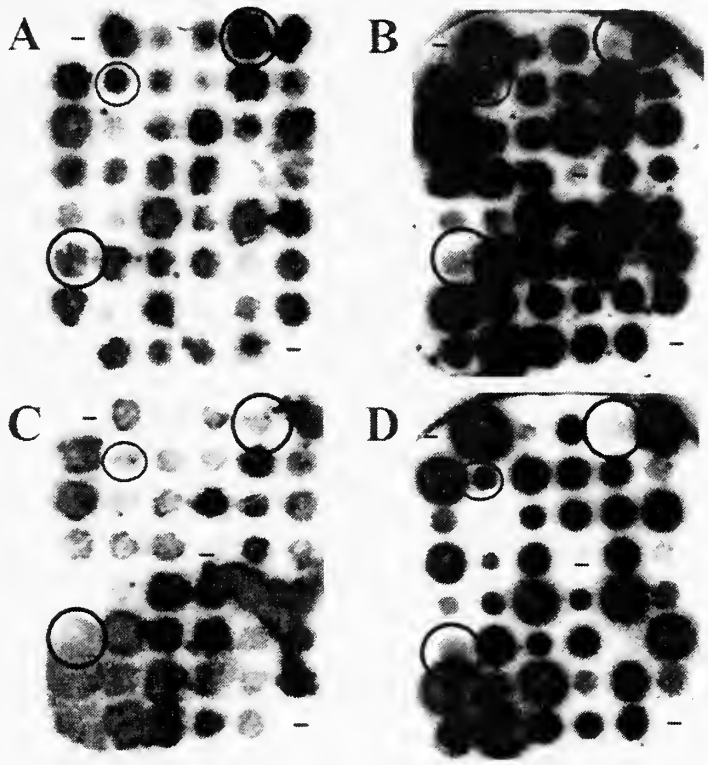

Figure 2. Colony hybridization of a pool of 45 signature-tagged mutants of *S. choleraesuis* with probes from the Input pool (**A**) and Recovery pools (**B,C,D**). The probes were (^{32}P) dCTP-labeled signature tags amplified by PCR from total DNA template from the inoculum bacteria (Input pool **A**) and bacteria recovered from the mesenteric lymph node 4 days after inoculating pigs orally (**B**: bacteria pooled from two pigs) or intraperitoneally (**C, D**). The large circles (mutants A5 and F1) indicate mutants with candidate pathogenicity genes tagged and therefore inactivated (attenuated mutants); these mutants were detected in the Input (**A**) but not Recovery pools (**B,C,D**). The mutant marked with the small circle (B2) was less likely to be an attenuated mutant because it was lacking in only one of the Recovery pools, a pig inoculated intraperitoneally (**C**). The short lines (–) indicates negative controls where the wells in the microtiter dish of the arrayed mutants had culture broth only. Based on this control, Blot **A** was contaminated in the center spot during transfer; we have since made the sterility protocol more robust. Colonies (mutants) are named by position on the blots: row (A-H) and column (1-6).

To confirm attenuation of these three attenuation candidates, competitive indices were determined in vitro and in vivo. Results in Table 1 indicate that all three candidate mutants were attenuated in pigs even though two of the three (A5 and B2) may have had an advantage in culture broth (indices over 1). The competitive indices of the two negative controls (not candidates for attenuation; C4 and G3) approach 1.0 which is consistent with their presence in the Recovery pool.

Table 1. Competitive indices of five mutants from a pool of forty-five mutants screened for colonization in pigs.

		Competitive Indices[2]			
	Recovery		In vivo		Attenuation
Mutant	Pool[1]	In vitro	Lymph node	Intestine	in pigs[3]
A5	--	1.6245	0.0016	0.0010	+
B2	--	3.6875	0.0372	0.1521	+
F1	--	0.4450	0.0010	0.0015	+
C4	+	0.4825	0.8278	0.9043	--
G3	+	0.6851	0.5761	0.5239	--

[1] Mutants were either recognized (+) or not (–) by hybridization in colony blot assays of the Input pool probing with a ^{32}P-labeled, PCR amplicon of the signature tags made from the DNA of *S. choleraesuis* in Recovery pools from inoculated pigs.

[2] Indices were the ratios of mutant to wild type bacteria after mixed challenge growth that started with an inoculum of a 1:1 mixture of mutant and wild type bacteria in broth (in vitro) or in pigs (in vivo; oral inoculation with *Salmonella* culture isolation 3 days later).

[3] If the in vivo index was less than < 0.4, the mutant is attenuated (+); otherwise it is not attenuated (--) (Darwin & Miller, 1999).

A 3 to 8 kb segment of the tagged gene in the three attenuated mutants has been cloned into pUC18. The 5' end of one clone (mutant A5) has been sequenced, and the GenBank searched for homologes. The sequence is complementary with *hilA* of *S. typhimurium* and *S typhi,* a positive transcriptional regulatory gene that controls expression of invasion genes (Bajaj et al., 1995, 1996).

Discussion

Our signature tag mutagenesis protocol is validated by the identification of candidate attenuated mutants from the test pool of 45 random mutants and the confirmation of attenuation by competitive indices from mixed challenge growth. Interestingly, mutant B2 was the least attenuated (higher competitive index than the other two attenuated mutants) and it was the only one of the three attenuated mutants that was not absent in all Recovery pools. More mutants will need to be tested to confirm if this correlation holds and how sensitive competitive indices are for the level of attenuation.

The functional genomic investigative protocol is also suppported by the identification of the first gene associated with attenuation as *hilA,* a recognized virulence gene in mice studies. However, in

contrast to *S. typhimurium* in mice where only enteric infection is attenuated with mutation (Penheiter et al., 1997), *hilA* appears to have roles in both enteric and systemic invasion in pigs (the mutant failed to colonize pigs inoculated orally or intraperitoneally). Recent investigations of *hilA* function in the mouse also suggests a more complex role than previously suspected (Murray & Lee, 2000).

Our functional genomics protocol investigates virulence factors in the natural host, avoiding the problems of transferring mechanistic data between host species which can be incorrect because of host specific differences (Tsolis et al., 1999). Additional attenuated mutants will be isolated to identify other genes involved in *S. choleraesuis* virulence; some will be genes associated with virulence in other systems, some will be previously identified genes not known to have roles in pathogenesis, and others will be novel genes without previous characterization. We are extending our investigations to *S. typhimurium*. The ultimate goal is to investigate the mechanisms of *Salmonella* virulence genes and products and their functions in swine infections. These mechanisms are targets for directed, molecular methods (such as DNA vaccines) to control salmonella in pigs.

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