Midwest Swine Nutrition Conference Proceedings

Indianapolis, Indiana — August 31, 2004

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

PURDUE UNIVERSITY

THE OHIO STATE UNIVERSITY

MICHIGAN STATE UNIVERSITY
FOUNDED 1855

UK

Sponsors
2004 Midwest Swine Nutrition Conference

Appreciation is expressed to the following companies who have financially supported the Midwest Swine Nutrition Conference. Their support is essential for the continuation of this activity

Ajinomoto Heartland LLC
Akey
Alltech
APC Company, Inc.
BASF Corporation
Degussa Corporation
DSM Nutritional Products, Inc.
Elanco Animal Health
Hubbard Feeds, Inc.
International Ingredient Corporation
Land O'Lakes/Purina Mills
Novus International, Inc.
Pig Improvement Company
Pioneer, A DuPont Company
Prince Agri Products, Inc.
United Feeds, Inc.
Zinpro Corporation

Appreciation is also expressed to the staff at the Indiana Pork Producers Association for their efforts in hosting committee meetings, helping to administrate the location of the meetings, and in registration efforts. Their web address is: www.inpork.org

INDIANA PORK

CONTENTS

Feeding the Modern Sow—Do We Really Know How?

Donald C. Mahan
Department of Animal Sciences
The Ohio State University
Columbus, OH 43210
Phone: 614-292-6987
E-mail: mahan.3@osu.edu

Summary

The modern high producing sow produces more pigs and more milk than the traditional 3-way cross used only a few years ago. The modern gilt has less body fat, but more muscle and bone at breeding. Gilts need to be bred at the proper body condition and weight to achieve longevity in the herd. Gilt weights of 135 to 145 kg, backfat thickness > 17 mm (last rib), and provision of higher gestation protein levels during parity 1 and 2 and then lowering the gestation protein fed to older sows is recommended. Providing the gestating sow with the higher protein and mineral lactation diet prior to farrowing will allows a better transition to the lactation period. Flushing gilts and sows to maximize ovulation and conception rates should enhance sow retention in the herd.

Introduction

Within the various feeding phases of swine production, the breeding herd and in particular the lactating sow is the most abused area where feeding programs and diet formulation are not well understood and errors are made. The high annual sow replacement rate and high cost of these animals lowers the profitability of the enterprise because of poor feeding practices. Although this can be attributed to several factors, the major effects are anestrus, poor conception rates, and foot and leg problems, each resulting in the culling of animals. When we consider that most of the published sow research in the United States was conducted historically with the three-way cross and largely with first parity animals, we are grossly behind the Europeans where they have been more successful in keeping sows for several parities. Sow research is difficult to conduct and inherently has many uncontrollable variables that contribute to much of the data not having value. Coupled with the fact that current sow genotypes have changed to high producing sow lines makes much of the published research of minimal value. The modern sow is capable of extremely high production capacity. It is not unusual today for sows to farrow 12 or more pigs, to wean 10.5 to 11.0 pigs per litter, with average pig weaning weights of 6.5 kg at 17 to 19 days of age. The modern sow is a high milk producer, albeit for a short period (e.g., ≤ 3 weeks), capable of producing as much milk as a dairy cow when expressed on a body weight basis. Traditional methods being used to feed these sows are out-dated and undoubtedly contribute to the high gilt replacement rate in many commercial sow herds. This brief review will outline some of the biological principles in feeding gilts and sows with suggestions on modifying feeding programs.

Principals of Sow Growth and Body Composition

We first need to establish that there are some biological principals that guide the sow's reproductive life that if not followed will result in culling her from the herd. The first is that she will attempt to maintain her life (maintenance) thus modifying her productivity and body composition to attain this goal. The second factor is that the sow has a genetic capacity to produce a certain quantity of muscle and bone,

and until this genetic capacity is completed she will attempt to fulfill this demand at the expense of other functions. The third principal is that the sow has a genetic capacity to ovulate a certain number of eggs and to produce a certain quantity of milk. Thus the modern sow produces both a large litter size and has a large milk production capability. Milk composition and production are largely resilient to change, with body composition being modified to meet these biologic demands. Although nutrition and other factors can influence the outcome of these biological effects, the swine producer must understand the genetic capability of the animal's within their herd and their needs to achieve maximum output.

Because the swine industry has found that high producing lines of pigs perform best under confinement conditions, the role man has in understanding what these needs are with different types of facilities and environmental conditions is critical to her future reproductive performance. The reason research is needed is that we do not completely understand the desired nutrient and environmental factors that will result in maximum reproductive performance and sow longevity.

With the advent of new sow lines, genetically capable of producing more pigs and yielding more milk than the traditional sow lines once used, sows are heavier at physiological maturity, and body fat is lower at each phase of development. Consequently, many sows are lost from the herd because we have failed to modify our feeding and dietary formulation practices with these new genotypes.

Importance of body fat in estrus onset

Questions arise if there is a minimum body fat content of the young gilt that is essential for these new genotypes to demonstrate estrus, and at what age should we breed gilt's to attain maximum life performance responses.

One of the first studies to demonstrate this effect was reported by Newton et al. (1992). These workers fed three sets of gilts from approximately 4.5 months of age to breeding either at: 1) full feed, 2) the same diet at 75% or 3) the diet at 50% of the full fed group. They also evaluated the onset of estrus either by behavioral responses (back pressure) or analyzing serum progesterone (> 1 ng/mL), the latter measurement determining if ovulation occurred. Their results presented in Table 1 demonstrated that as the gilt becomes heavier in body weight and had a greater amount of body fat it was more difficult to detect estrus by conventional behavioral characteristics. However, by chemically analyzing serum progesterone, a higher percentage of the heavier weight animals had indeed ovulated. Body fat content at the time of ovulation clearly differed between the groups at estrus onset, but the age when estrus occurred was relatively similar between groups and clearly was not dependent on body fat content. These results show that gilt age is the predominant factor that determines when ovulation occurs but if feed is restricted, particularly at < 75%, such that growth rate is reduced, estrus and ovulation can be curtailed. Because body fat content cannot be determined practically on commercial farms, the use of backfat thickness in this study was measured as an index of body fat. Backfat thickness in the study showed the same effect as body fat content.

These results are largely confirmed by the data of Zier et al. (2004) where a set of gilts were all fed the same diet. They grouped the gilts by backfat thickness and weight and determined the age at first service. Their results in Table 2 demonstrated that gilts with lower backfats exhibited estrus as soon or sooner than gilts that had greater backfats and heavier in body weight. When the data are expressed on a body weight basis (Table 3) it appeared that the gilts with more backfat were somewhat older at first service.

The combined results from these studies suggest that as gilts attain more body weight and deposit excess body fat, it may become more difficult to detect estrus. However, both excessive and inadequate body fat could be detrimental to future breeding performance.

Effect of backfat at first service on subsequent reproductive performance

When the first parity gilts of differing backfats were bred and reproductive performances measured, the results demonstrated (Table 2 and 3) that litter size (total and live pigs) was not seriously affected by the low backfat thickness (Zier et al., 2004). Continued successful reproductive performance of these animals with the lower backfats is, however, questionable. This was demonstrated in another study where backfat thickness collected at 100 kg body weight was measured and reproductive performance was subsequently evaluated over a 4 parity period. The results demonstrated a lower percentage of gilts

the wall shaft of bones, thus increasing the reservoir of these minerals in the body (Maxson et al., 1983). Sows of higher production have greater dietary needs for minerals, particularly Ca and P than sows with lower productivities (Maxson et al., 1982). Body mineral composition has demonstrated that over a 3-parity period sows of high productivity had a 15 to 17% lower body Ca and P content. Unpublished data from our station has also demonstrated that approximately 40 to 50% of the total minerals (macro and micro) retained by developing fetus's occurred during the last 14 days of gestation. The mineral demands of the high producing sow are high, explaining the high occurrence of structural problems encountered on many swine farms.

Feeding programs

Phase feeding of weanling pigs and grower finisher pigs is common place. It will become more common to feed sows in the same manner as with the other phases of swine feeding practices. Sows should be fed to their genetic capability. This means that body condition at each production phase should be monitored and feed provided to the animal adjusted accordingly. When the diet does not meet the sows genetic capacity to produce fetus's or milk, she will divert nutrients from body tissue to meet these demands. Figure 4 presents a method of feeding sows during gestation and lactation that should maximize reproductive performance.

Literature cited

Close, W. H. and D. J. A. Cole. 2000. Nutrition of Sows and Boars. Nottingham University Press. Chpt 12.

Clowes, E. J., F. X. Aherne, A. L. Schaefer, G. R. Foxcroft, and V. E. Baracos. 2003. Parturition body size and body protein loss during lactation influence performance during lactation and ovarian function at weaning in first-parity sows. J. Anim. Sci. 81:1517-1528.

Clowes, E. J., I. H. Williams, V. E. Baracos, J. R. Pluske, A. C. Cegielski, L. J. Zak, and F. X. Aherne. 1998. Feeding lactating primiparous sows to establish three divergent metabolic states: II. Effect on nitrogen partitioning and skeletal muscle composition. J. Anim. Sci. 76:1154-1164.

Mahan, D. C. and L. T. Mangan. 1975. Evaluation of various protein sequences on the nutritional carryover from gestation to lactation with first-litter sows. J. Nutr. 105:1291-1298.

Mahan, D. C. and E.A. Newton. 1995. Effect of initial breeding weight on macro- and micro-mineral composition over a three-parity period using a high-producing sow genotype. J. Anim. Sci. 73:151-158.

Mahan, D. C. 1998. Relationship of gestation protein and feed intake level over a five-parity period using a high-producing sow genotype. J. Anim. Sci. 76:533-541.

Maxson, P. F. and D. C. Mahan. 1983. Dietary calcium and phosphorus for growing swine from 18 to 57 kilograms body weight. J. Anim. Sci. 56:1124-1134.

Maxson, P. F. and D. C. Mahan. 1986. Dietary calcium and phosphorus for lactating swine at high and average production levels. J. Anim. Sci. 63:1163-1172.

Newton, E.A. and D. C. Mahan. 1992. Effect of dietary feed intake during late development in puberty onset and resulting body composition in crossbred gilts. J. Anim. Sci. 70:3774-3780.

Newton, E. A. and D. C. Mahan. 1993. Effect of initial breeding weight and management system using a high-producing sow genotype on resulting reproductive performance over three parities. J. Anim. Sci. 71:1177-1186.

Swick, R. W., and N. J. Benevenga. 1977. Labile protein reserves and protein turnover. J. Dairy Sci. 60:505-515.

Zier, C. E., N. H. Williams, and B. Wolter. 2004. Gilt growth may affect longevity; new attrition concept introduced. (cited by Lundeen in Feedstuffs March 22. Vol 76:(No.12) 9.

Table 1. Effect of feed intake on puberty onset in gilts

	Feed intake level, % of full feed		
Item	50	75	100
No. of gilts	35	35	35
Percent of gilts demonstrating ovulation			
Behavioral [a]	57	60	45
Hormone detection [b]	77	88	91
Age at estrus detection			
Behavioral [a]	223.0	223.8	227.5
Hormone detection [b]	224.8	219.5	218.7
At estrus detection			
Backfat, mm	17	21	26
Body fat, kg	31	39	47
Body weight, kg	108	123	140

Source: Newton and Mahan, 1994.
[a] Positive response to back pressure (herdsman).
[b] Plasma progesterone (> 1 ng/mL) concentration.

Table 2. Effect of backfat thickness (first mating) on first parity reproductive performance

Item	P_2 Backfat thickness (P_2 area), mm					
	<12	12-14	14-16	16-18	18-20	>20
No. of gilts	124	361	431	364	231	163
Mating measurements						
Backfat, mm	11.4 [a]	13.5 [t]	15.5 [c]	17.4 [d]	19.3 [e]	22.6 [f]
Body weight, kg	143 [a]	154 [b]	162 [c]	162 [c]	162 [c]	168 [c]
Age (first service), day	222	225	233	231	234	237
Reproductive performance						
Total born/litter, no.	11.5	11.9	11.6	11.8	11.7	12.1
Live born/litter, no.	10.3 [c]	10.8 [a]	10.6 [a]	10.8 [ab]	10.8 [ab]	11.3 [b]
Gilt removal, %	6.5 [a]	2.2 [t]	3.0 [a]	3.8 [ab]	4.3 [ab]	1.8 [b]

Source: C.E. Zier, N.H Williams, B. Wolter. 2004. (cited by Lundeen, Feedstuffs, March 22 (76; No. 12; pg 9).

[abcde] Superscripts on the same row that differ are significantly different, $P < 0.05$.

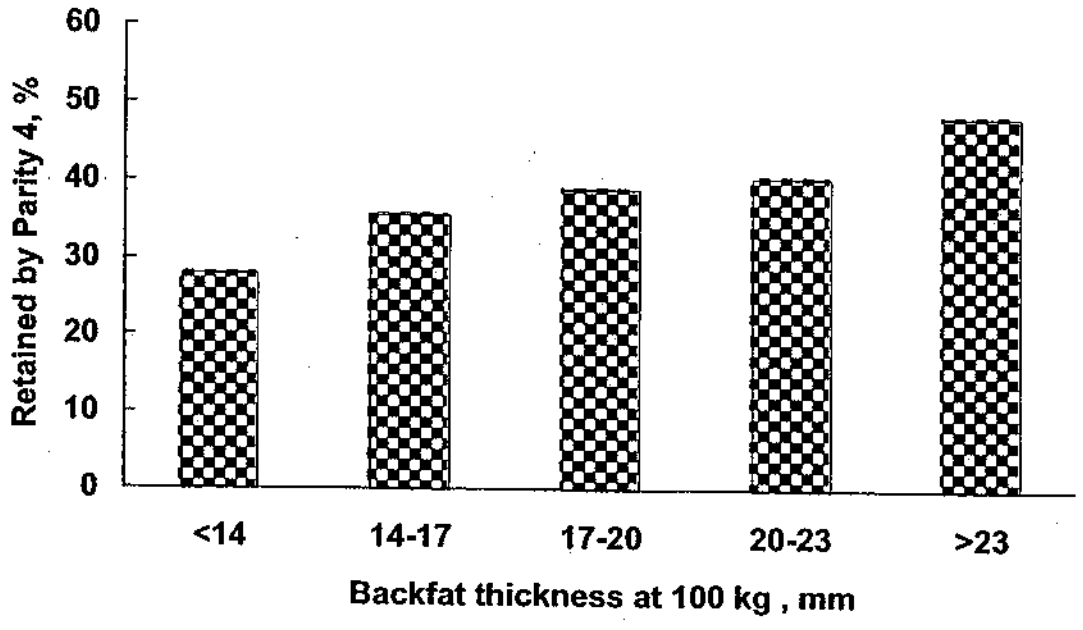
Table 3. Effect of body weight (first mating) on first parity reproductive performance [a]

Item	Body weight, kg				
	<136	136-147	147-158	158-170	>170
No. of gilts	92	136	181	124	157
Mating measurements					
Backfat, mm	13.9 [a]	15.1 [b]	16.2 [c]	16.8 [cd]	17.5 [d]
Body weight, kg	132 [a]	146 [b]	156 [c]	165 [d]	184 [e]
Age (first service), day	205 [a]	218 [b]	229 [c]	236 [d]	250 [e]
Reproduction performance					
Total born, no.	11.2 [a]	11.8 [ab]	11.6 [ab]	12.2 [b]	11.7 [ab]
Live born, no.	10.5 [ab]	10.6 [ab]	10.6 [a]	11.3 [b]	10.4 [a]
Gilt removal, %	1.6	1.8	5.3	5.6	5.1

Source: C.E. Zier, N.H. Williams, B. Wolter. 2004. (cited by Lundeen, Feedstuffs, March 22 (76; No. 12; pg 9).

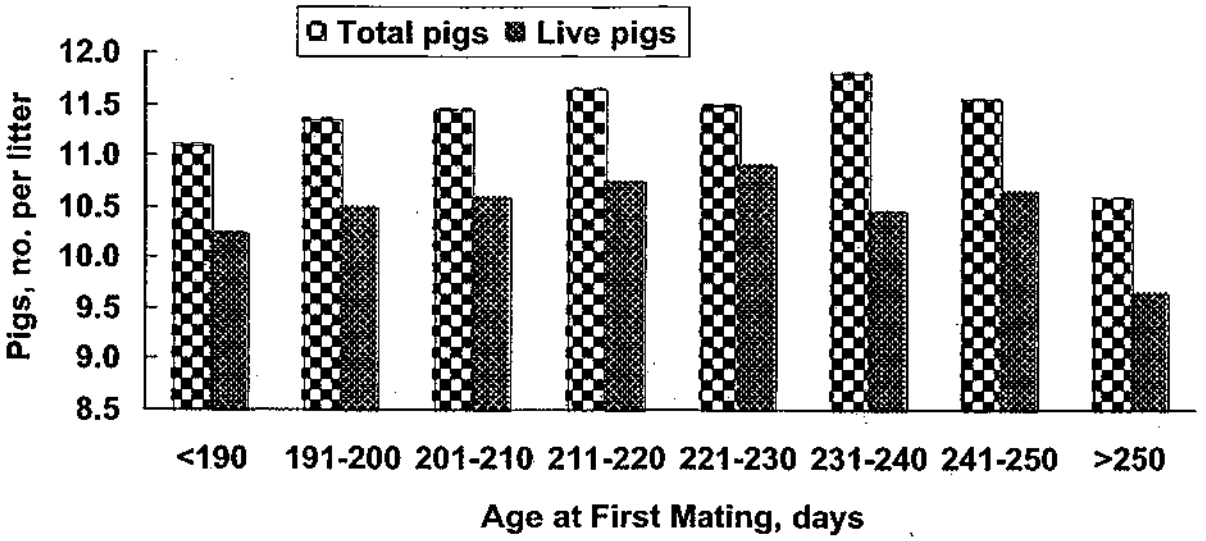
[abcde] Superscripts on the same row that differ are significantly different, $P < 0.05$.

Figure 1. Backfat thickness (100 kg body weight) on sow retention by parity 4



Source: Agr. Inst. of France (1987)

Figure 2. Effect of age of first mating on sow reproductive performance



Source: Pig International (Dec., 2003)
A total of 12,929 litters from Camborough 22 sows were evaluated.

Figure 3. Body weight and body compositional changes of sows during the reproductive cycle

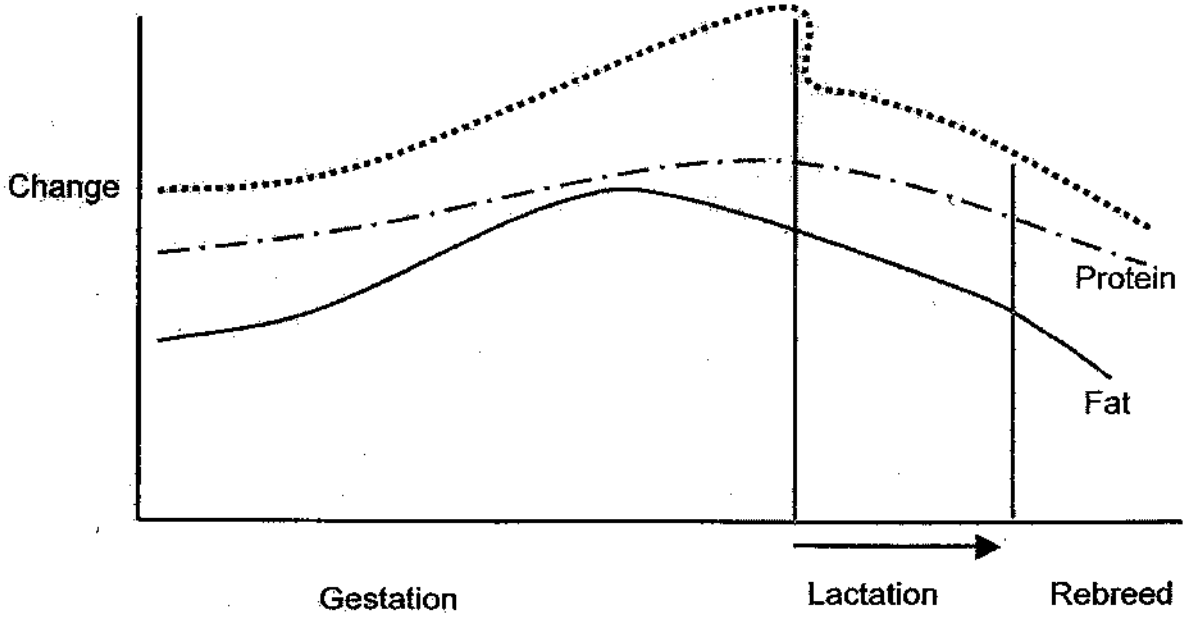
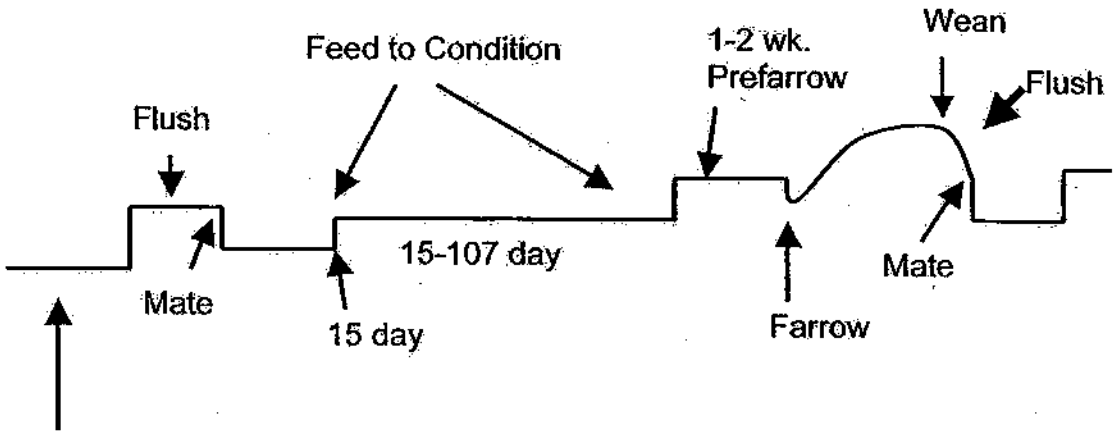


Figure 4. Feeding strategy for reproducing sows



Source: Cole and Close, 2000

Table 5. Leanness at selection time on sow longevity and performance

Item	Low BF <13	Med. BF 14-16	High BF >17	P < .xx
Litters/sow	2.81	3.47	3.75	.05
Total pigs born	27.47	34.85	37.55	.05
Pig birth wt., kg	1.51	1.34	1.32	.05
Pig wean wt., kg	6.87	6.71	6.47	.05
Total pigs weaned, no.	21.91	27.63	30.08	.01

Source: Guaghan et al., 1995.

Table 6. Protein carryover in the sow from gestation to lactation

Item	Gestation:	9 % CP		13 % CP		18% CP		SEM
	Lactation:	12%	18%	12%	18%	12%	18%	
Sow feed, kg/day		4.2	6.2	4.8	6.5	5.9	6.2	0.7
Litter gain, kg		26.7	36.2	28.2	36.1	32.1	35.4	0.5
Pig wt., kg		5.6	6.7	5.9	7.3	6.4	6.7	0.1

Source: Mahan and Mangan, 1975.

Energy Systems for Swine: A Critical Review of DE, ME and NE

John F. Patience, A. Denise Beaulieu, Ruurd T. Zijlstra, Temi Oresanya and Ryan Mohr
Prairie Swine Centre Inc.
Saskatoon, SK
CANADA S7H 5N9
306-667-7442
john.patience@usask.ca

Introduction

Energy systems are employed in animal nutrition for two fundamental reasons. The first is to define, in quantitative terms, the energy content of an ingredient, or blend of many ingredients, to serve financial purposes including pricing and quality determination. The second is to define, in quantitative terms, the requirement for, and use of, energy by the pig for maintenance and productive purposes (eg. growth, lactation, reproduction) to serve as the basis for diet formulation and the development of feeding programs.

Interest in energy systems, and their application to practical swine nutrition, has increased in recent years. There are many reasons for this. In the case of our own research, we have come to realize that in order to adequately and effectively apply our increasing knowledge of amino acid metabolism, there must be a concomitant increase in our understanding of energy. We do not believe that the level of sophistication applied to amino acid requirements is matched by that of energy. As our industry seeks to further reduce costs and increase revenues, there is increased interest in diets designed to achieve desired carcass outcomes; energy is as much involved in carcass composition as are amino acids. Furthermore, defining the value of various ingredients, in terms of their true value to the pig, demands systems that are more rigorous than the Digestible Energy (DE), or Metabolizable Energy (ME) systems. There is also increasing recognition of the inherent variability in the nutrient composition of ingredients, something that must become more entrenched in purchasing practices and in diet formulation. The globalization of our industry has meant that nutritionists in North America have become exposed to different energy systems than those routinely employed locally, and this exposure has led to questions about the adequacy and suitability of DE and ME.

Without question, the Net Energy (NE) system has gained wider acceptance in Europe than it has in North America. There are many reasons for this regional difference. Higher feed costs have motivated the European livestock and poultry industries to adopt technologies that reduce the cost of feed. Many European countries are net importers of feedstuffs, and they have sought to minimize their imports and their purchasing costs to the greatest possible extent. Given the nature of the ingredients used in Europe, the advantage of a net energy system is clear. Although we may be guilty of over-simplifying the situation somewhat, we believe that in Europe, the focus of the pork industry has been on feed costs, whereas in North America, the greater focus has been on animal performance. This may explain, in part, the more rapid adoption of a NE system in Europe compared to North America, because without doubt, the net energy system is superior for this purpose.

We refer to "one of the NE systems" since there are a number in use in Europe. The most prominent of these were developed at INRA under the outstanding leadership of Dr. Jean Noblet (Noblet, 2000), and at the Dutch Central Bureau for Livestock feeding (Centraal Veevoederbureau – CVB). Whilst there is only one DE or one ME system in place in North America, (NRC,1998), this is not the case in Europe.

Ideal Energy System

The ideal energy system should result in predictable animal performance and carcass composition. It must be equal in its suitability for ingredient evaluation and for diet formulation. Obviously, the values for each individual ingredient must be additive when blended into a diet and it should be easy to determine and/or estimate the energy values of ingredients and feeds. Finally, the energy values in an ideal system should be accurate, precise and repeatable.

It is easy to conclude that none of the energy systems available today completely fulfill these standards. As we will discuss later in this presentation, each system has its advantages and disadvantages. Perhaps what is most critical is a clear understanding of the assumptions that are necessary in the use of any of the energy systems. With this in mind, it will be possible to achieve the greatest success in diet formulation within a given system.

Existing Energy Systems

Functionally, energy can be classified according to how it is used by the pig. Whittemore (1993) has summarized this very well, as shown in Figure 1. The gross energy, measured by complete combustion of the feed sample, is presented to the pig, and a portion is excreted as energy in the faeces; the residual is DE. Of the DE, a portion is lost in the urine and in gaseous excretions from the stomach; the residual is ME. Of the ME, a certain portion will be used for maintenance, including basal metabolism and the heat of digestion. A further portion of the ME is lost as heat due to that used for cold thermo genesis or for the "work" required to support growth, reproduction and/or lactation. If there is any energy left over, it is deposited on the body of the pig to support growth, used for the production of milk in lactating sows or retained as the products of conception in pregnant sows. As can be seen in Figure 1, there is a considerable quantity of energy "lost" between that which is called DE or ME, and that retained by the pig. The so-called "heat increment" has also been called dietary induced thermo genesis, the thermic effect of feed and specific dynamic action (Yen, 1997). The classical definition of the different energy systems is presented in Figure 2.

The sole difference between DE and ME is urinary and gaseous energy losses. Generally, gaseous losses are ignored as insignificant and urine is rarely measured in energy balance studies. As a consequence, most ME values have been derived arithmetically from DE values, which have been measured directly. The benefit of the ME system over DE is therefore moot. Following are various equations which have been used to convert DE to ME:

(1) ME = DE (0.998 – (0.002*CP))
(Noblet et al., 1989)

(2) ME = DE (1.003 – (0.0021*CP))
(Noblet and Perez, 1993)

(3) ME = DE (1.012 – (0.0019*CP)
(May and Bell, 1971)

Variation in any of the components related to maintenance, to digestion or to the "work" of growth/lactation/reproduction brings in to play a fluctuation in animal performance that will not be predicted by either DE or ME. The Net Energy system was developed to address this issue, and in particular to recognize the well-known differences in the energetic cost of utilizing dietary protein, fibre, lipid or carbohydrate. This is commonly referred to as the "heat increment." It varies substantially among the various classes of ingredients commonly used in pork production (Table 1). Ingredients that are high in crude protein or fibre decline in relative energy content when compared to ingredients that are high in fat or carbohydrate. For this reason, Noblet et al. (1994) estimates that the NE of tallow is very similar to its DE or ME, while the NE of soybean meal is much lower than its DE or ME.

The other factor that affects the "net energy" of an ingredient, or a feed, is the composition of growth, as well as the relationship between maintenance and growth. The efficiency with which the pig uses energy for lean gain differs from the efficiency of energy used for lipid gain. The energetic efficiency of protein gain has been estimated by the NRC (1998) as 10.6 kcal ME/kg compared to that for lipid gain of 12.5 kcal ME/kg. The difference in energetic efficiency of protein versus lipid is exaggerated by the much greater quantity of water associated with protein gain as compared to lipid. This problem can be looked at another way. Various studies, reported in the 1970's and 1980's, estimated the efficiency with which ME was converted to NE. All reported that the efficiency with which ME is used for protein

deposition was less than that for fat deposition, with mean coefficients of 0.52 and 0.72 for protein and fat deposition, respectively. Since the maintenance requirement of the pig can be estimated at 106 kcal/kg $BW^{0.75}$ (NRC, 1998), it becomes immediately clear that in order for the net energy system to work well, the ultimate use of the energy must be known. We believe it is fair to say that such is not the case under most commercial, or even research, conditions.

Other factors may affect NE. Because genotype is one of the major determinants of the composition of gain, it will affect the NE of the diet (Patience et al., 2001). Furthermore, it is also known that genotypes vary in their maintenance requirements (Noblet et al., 1999), although the differences are not large. The level of activity may also be a variable, depending on the nature of the housing system and the pig's social environment. Collin et al. (2001a,b) suggested that the energetic cost of activity could vary from 47 to 59 kcal/kg $BW^{0.60}$.

Another problem that is not addressed by either DE or ME is the differing utilization of dietary components (eg. fat, fibre) by pigs of different ages (Noblet, 1996). These differences are substantial and cannot be ignored. Simply stated, all of the energy systems are affected by what might be called animal factors, with age being the most obvious. One can reasonably predict then, that irrespective of the energy system adopted, at least two standards will be required – one for growing pigs and lactating sows and one for gestating sows. There is uncertainty as to the suitability of energy values that were determined using the growing pig being used for weanling pigs.

Other Energy Systems

Emmans (1994) has proposed an alternative energy system that he has termed "effective energy." It is based on predicting the heat increment of a diet from its crude protein content and considering the extra-caloric effect of dietary lipid deposited directly as carcass fat. The resulting equation predicts effective energy from the ME value of an ingredient:

$$EE\ (KJ/kg) = 1.17\ ME_n - 4.29\ CP - 2.44$$

(Emmans, 1994)

where ME_n is the ME value measured or estimated at zero nitrogen retention and CP is the ingredient crude protein content.

The Danes recently revised their energy evaluation system. It remains based on their well-known FU (feed units) system, which is now going to be based on the physiological energy value of an ingredient, or on its ATP equivalents. In this respect, the Danes have rejected the net energy system, and indeed, any system based on bomb calorimetry. Their new system, introduced in 2002, now has separate energy values for gestating sows, as distinct from lactating sows and growing pigs. Under the new Danish system, the following equation estimates the FU for a growing pig (and lactating sow):

$$FU_{gp} = 9.9*RDCP + 31.7*RDCF + factor*IDC + 7.0*FC - 28*EUDMi$$

where RDCP is real digestible crude protein, RDCF is ideal digestible crude fat, IDC is ileal digestible carbohydrate, FC is fermentable carbohydrate and EUDMi is enzyme undigested dry matter measured at the ileum.

This new system has increased, in relative terms, the energy value of barley, wheat and oats, but lowered the energy value of soybean meal, fish meal and rapeseed meal, as compared to the previous Danish energy system. In this respect, the new Danish system has had the same impact as net energy, that is to say it has decreased the relative value of protein sources and increased the relative value of low protein ingredients.

Other Issues

The livestock industries (pigs, poultry, cattle) are slowly acknowledging that great variability exists in the energy content of common feed ingredients (Fairbairn et al., 1999). Table 2 summarizes what is currently known in this regard. All of the feed ingredients commonly used by the pork industry vary much more in energy content that most of us wish to admit. The challenge is in finding a solution to this problem; certainly bushel weight, the ubiquitous trading standard, must be discarded since for other than extreme highs and lows, it has proven to be a poor indicator of actual value to the pig (Fairbairn et al., 1999). The problem of ingredient variability is clearly illustrated in Figures 3 and 4.

Another issue surrounding all of the energy systems is their current inability to quantify the impact

of feed additives or feed processing on the energy made available to the pig. This problem, like the one above, affects all of the energy systems equally. For example, Zijlstra et al. (2002) showed an effect of enzymes (Figure 5) and Oryschak et al., (2002) showed an effect of particle size on measured digestibility of energy.

Finally, there remain serious challenges to predicting animal performance in response to changes in dietary energy concentration, irrespective of the energy system employed. Levesque et al. (2001, 2002) attempted to improve the growth rate of weanling pigs through increases in dietary energy and was unsuccessful in every attempt. The animals utilized the experimental diets effectively, as feed efficiency responded to changes in dietary energy concentration as expected. Oresanya et al (2004) observed that NE was not superior to DE in predicting the weanling pig's response to changes in dietary energy concentration.

Conclusions

It is difficult to draw any conclusion other than a much greater understanding of energy metabolism in the pig is required if diet formulation is to rise to the next level of sophistication. Certainly, there is a sense of comfort with the status quo, but given the high cost of energy in the diet, there are compelling economic reasons to expect more of our energy systems. With greater pressure on the industry, vis a vis carcass composition and environmental impact, the need for improvement will grow in the future.

One might reasonably ask that if additional research on energy is required, why not utilize the best possible platform for that work? It may very well be that the NE system is not capable of achieving our expectations with respect to a dietary energy system. However, it has many theoretical advantages over the DE and ME systems. It is least affected by the nature of the diet and supports more economic use of feed ingredients. Although much more knowledge on the growth patterns of current genotypes is required before it can be applied successfully in commercial practice, it has the potential to address the differential efficiency of use of energy for different components of body weight gain. Before the net energy system can be applied more widely in commercial practice, a greater body of empirical data will be required to support conversion from the current DE and ME systems.

References

Collin, A., J. van Milgen, S. Dubois and J. Noblet. 2001a. Effect of high temperature on feeding behaviour and heat production in group-housed young pigs. Brit. J. Nutr. 86: 63-70.

Collin, A., J. van Milgen, S. Dubois and J. Noblet. 2001b. Effect of high temperature and feeding level on energy utilization in piglets. J. Anim. Sci. 79:1849-1857.

Emmans, G.C. 1994. Effective energy: a concept of energy utilization applied across species. Br. J. Nutr. 71:801-821.

Ewan, R.C. 2001. Energy utilization in swine nutrition. In: A.J. Lewis and L.L. Southern (eds.) Swine Nutrition. p 85-105. CRC Press LLC, Boca Raton, FL.

Fairbairn, S. L., J.F. Patience, H.L. Classen and R.T. Zijlstra. 1999. The energy content of barley fed to growing pigs: characterizing the nature of its variability and developing prediction equations for its estimation. J. Anim. Sci. 77: 1502-1512.

Levesque, C., J.F. Patience, E. Beltranena and R.T. Zijlstra. 2001. Effect of site of weaning and diet DE content on performance of pigs to 56 d of age. J. Anim. Sci. 80 (Suppl. 2):81.

Levesque, C.L., J.F. Patience, E. Beltranena and R.T. Zijlstra. 2002. Impact of stocking density/group size on the response to changes in dietary energy content by weanling pigs from 25 to 53 days of age. J. Anim. Sci. 80 (Suppl. 2):60..

Lorschy, M.L., J.F. Patience, D.A. Gillis and S.L. Neis. 1998a. Comparison of lysine requirements based on animal performance, plasma urea nitrogen and nitrogen balance studies. Monograph No. 98-06. Prairie Swine Centre Inc., Saskatoon, Canada. 27 pp.

Lorschy, M.L., J.F. Patience, D.A. Gillis and S.L. Neis. 1998b. Defining the digestible threonine requirement based on protein deposition rate for pigs of a defined strain grown from 20 to 45, 45 to 75 and 75 to 120 kg body weight. Monograph No. 98-04. Prairie Swine Centre Inc., Saskatoon, Canada. 68 pp.

Lorschy, M.L., J.F. Patience, D.A. Gillis and S.L. Neis. 1998c. Defining the digestible tryptophan requirement based on protein deposition rate for pigs of a defined strain grown from 20 to 45, 45 to 75 and 75 to 120 kg body weight. Monograph No. 98-03. Prairie Swine Centre Inc., Saskatoon, Canada. 85 pp.

May, R. W. and J. M. Bell. 1971. Digestible and metabolizable energy values of some feeds for the growing pig. Can. J. Anim. Sci. 51: 271-278.

NRC. 1998. Nutrient Requirements of Swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

Noblet, J., H. Fortune, S. Dubois and Y. Henry. 1989. Nouvelles bases d'estimation des teneurs en energie digestible, metabolisable et nette des aliments pour le porc. INRA, Paris, France.

Noblet, J., H. Fortune, X.S. Shi and S. Dubois. 1994. Prediction of net energy values of feeds for growing pigs. J. Anim. Sci. 72: 344-354.

Noblet, J. 1996. Digestive and metabolic utilization of dietary energy in pig feeds: comparison of energy systems. In: Garnsworthy PC, Wiseman J, Haresign W, editors. Nottingham: Nottingham University Press.p 207-231.

Noblet, J., C. Karege, S. Dubois and J. van Milgen. 1999. Metabolic utilization of energy and maintenance requirements in growing pigs: effects of sex and genotype. J. Anim. Sci. 77: 1208-1216.

Noblet, J. 2000. Digestive and metabolic utilization of feed energy in swine: application to energy evaluation systems. J. Appl. Anim. Res. 17: 113-132.

Noblet, J. and J.M. Perez. 1993. Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. J. Anim. Sci. 71:3389-3398.

Oresanya, T.F., A.D. Beaulieu and J.F. Patience. 2003. The effect of dietary energy concentration and lysine:energy ratio on the growth performance of weaned pigs. 2002 Annual Research Report, Prairie Swine Centre Inc., Saskatoon, SK. pp. 25.

Oresanya, T.F., A.D. Beaulieu and J.F. Patience. 2004. Growth, body composition and nutrient deposition rates in weaned pigs fed diets with similar digestible but different estimated net energy content. J. Anim. Sci. (In press).

Oryschak, M.A., P.H. Simmins and R.T. Zijlstra. 2002. Effect of dietary particle size and carbohydrase and/or phytase supplementation on nitrogen and phosphorus excretion of grower pigs. Can. J. Anim. Sci. 82:533-540.

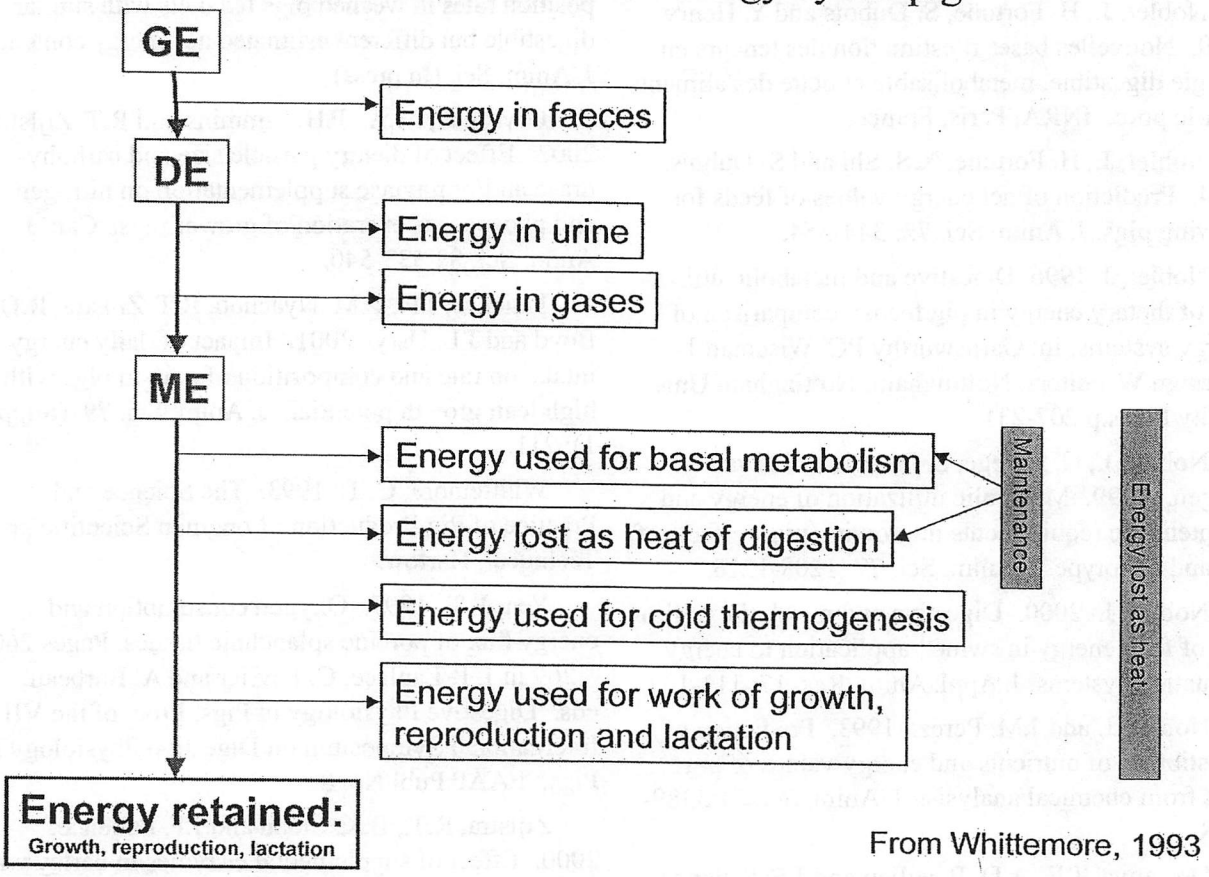
Patience, J.F., C.M. Nyachoti, R.T. Zijlstra, R.D. Boyd and J.L. Usry. 2001. Impact of daily energy intake on rate and composition of gain in pigs with high lean growth potential. J. Anim. Sci. 79 (Suppl. 1): 211.

Whittemore, C. T. 1993. The Science and Practice of Pig Production. Longman Scientific and Technical, Harlow.

Yen, J. T. 1997. Oxygen consumption and energy flux of porcine splanchnic tissues. Pages 260 – 269 in J. P. Laplace, C. Fevrier and A. Barbeau, eds. Digestive Physiology in Pigs. Proc. of the VII International Symposium on Digestive Physiology in Pigs. EAAP Publ No. 88.

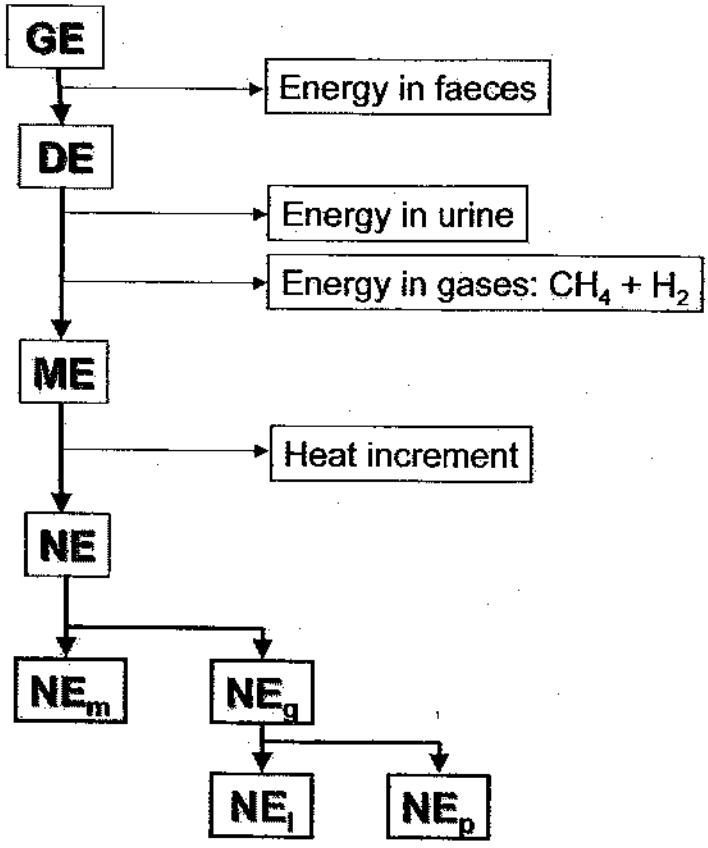
Zijlstra, R.T., B.K. Sloan and J.F. Patience. 2000. Effect of supplemental enzymes in barley with low, medium and high DE content fed to grower pigs. J. Anim. Sci. 78 (Suppl. 1):171.

Figure 1. Utilization of energy by the pig



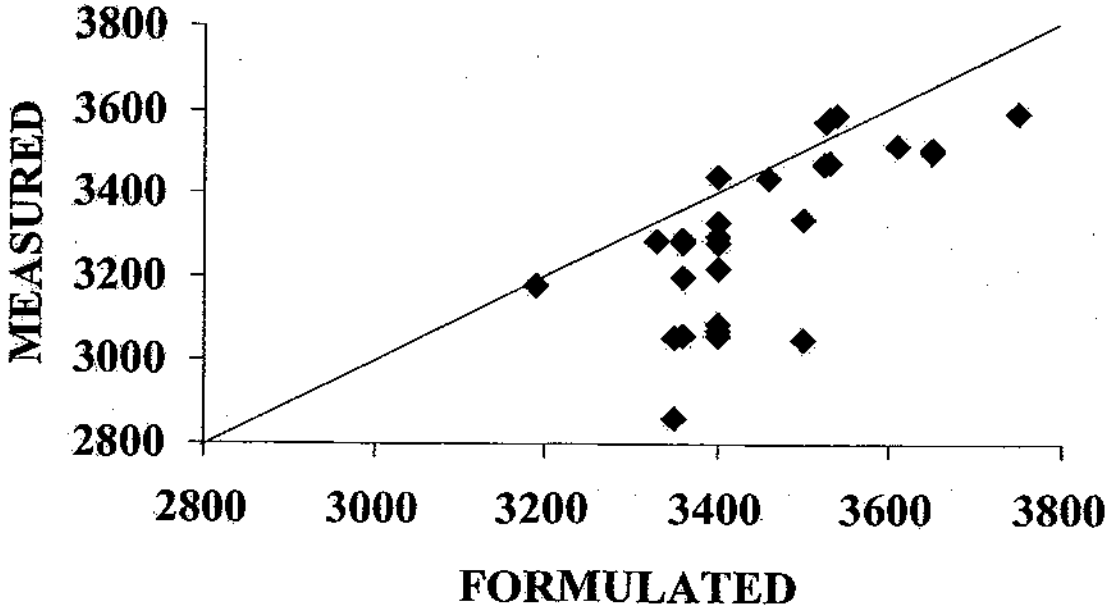
From Whittemore, 1993

Figure 2. Classical definition of energy systems



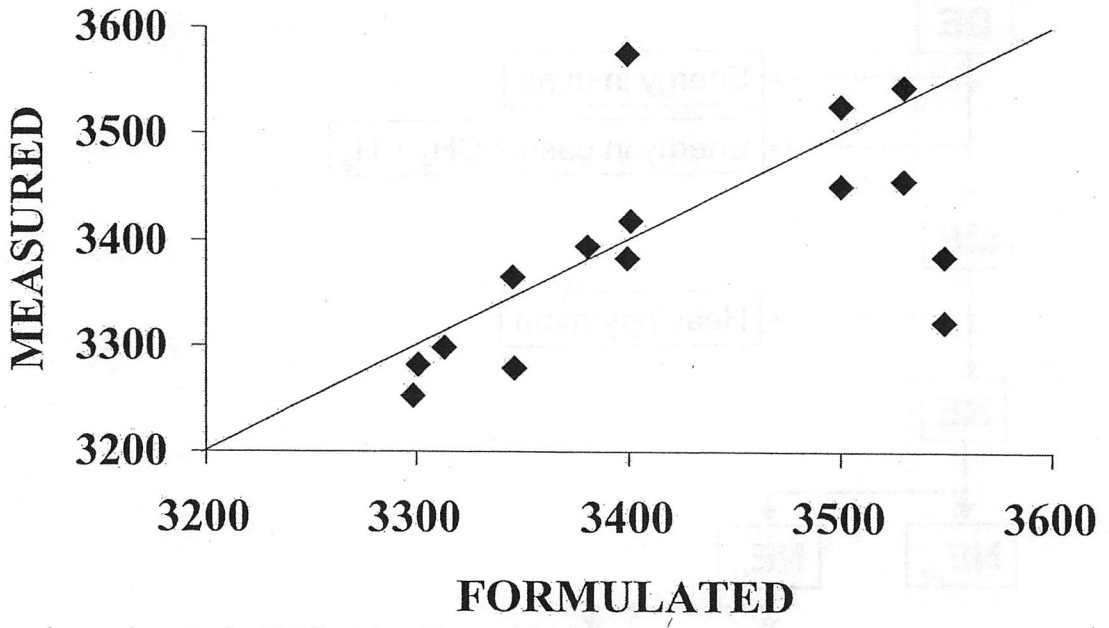
Adapted from Ewan, 2001

Figure 3. Formulated versus measured DE content (Kcal/kg) of diets fed to weanling pigs



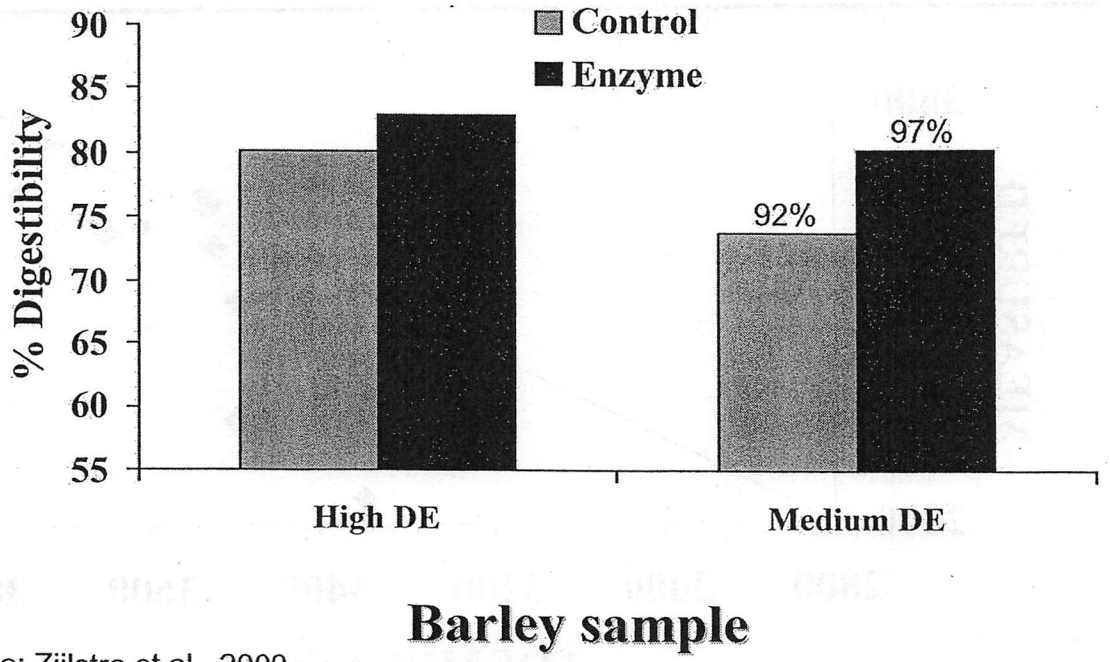
Sources: Levesque, 2001; Oresanya, 2003

Figure 4. Formulated Versus Measured DE Content (Kcal/kg) Of Diets Fed To Growout Pigs



Sources: Lorschy et al, 1998a,b,c; Ekpe, 2000

Figure 5. Use Of Enzymes To Reduce Variability In DE Content



Source: Zijlstra et al., 2000

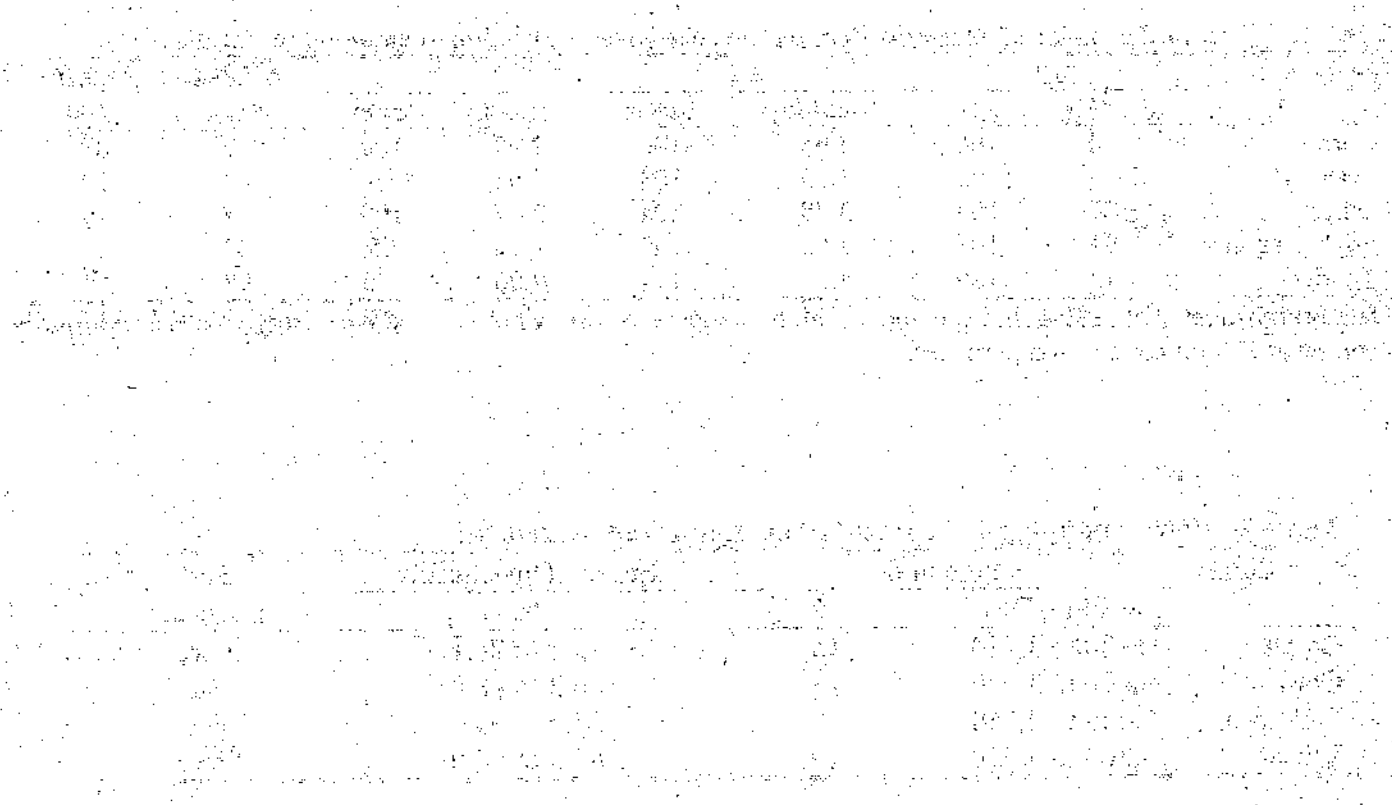
Table 1. Effect of the choice of energy system on the relative energy value of common feedstuffs

Ingredient	DE		ME		NE		NE/DE	NE/ME
	Mcal/kg	Index	Mcal/kg	Index	Mcal/kg	Index	*100	*100
Corn	3.78	100	3.65	100	2.97	100	79	81
Peas	3.88	103	3.75	103	2.64	89	68	70
Wheat	3.87	103	3.78	104	2.97	100	77	79
Soybean meal	3.91	103	3.65	100	1.93	65	49	53
Tallow	7.13	189	7.07	194	7.00	235	98	99

Data from Noblet et al., 1994. Index compares all ingredients to corn, whose respective energy value has arbitrarily been set at 100 for comparison purposes.

Table 2. Variation in the DE content of common feed ingredients

Ingredient	Range in DE		Energy Digestibility	Best
	Kcal/kg DM	%	%	Indicator
Barley	2,980 to 3,480	15	73.6 to 78.1	Fibre
Corn	3,490 to 3,970	13	86.3 to 88.8	Fat
Field peas	3,440 to 4,150	19	84.9 to 93.6	Fat
Wheat	3,360 to 4,050	19	80.3 to 88.0	Fibre



Acidification of Nursery Diets and the Role of Diet Buffering Capacity

M. C. Walsh, L. Peddireddi and J. S. Radcliffe

Purdue University
Department of Animal Sciences
West Lafayette, IN 47907
765-496-7718
jradclif@purdue.edu

Summary

Acidification of nursery diets has been investigated for many years as a possible means to minimize or eliminate post-weaning lag. A renewed interest in organic and inorganic acid addition to nursery pig diets has arisen out of concerns regarding the routine use of sub-therapeutic levels of antibiotics in nursery pig diets. This paper will not attempt to compare diet acidification with antibiotic use in swine diets. Instead it will discuss the known and proposed modes of actions of various organic and/or inorganic acids and the effect that dietary buffering capacity may have on pig performance and acidifier effectiveness.

Introduction

At the time of weaning the young pig's digestive system is immature and not yet capable of efficiently digesting a complex grain based diet. The microbial population within the gastrointestinal tract at weaning is very undiversified and unstable, making it susceptible to colonization by pathogenic microorganisms. Therefore, much attention has been placed on this early nursery period. The addition of organic acids to nursery pig diets have been shown to improve nursery pig performance (reviewed by Mroz, 2003). Proposed mechanisms of action include reductions or stabilization of gastric pH, alterations in gut microflora populations through bacteriostatic or bactericidal mechanisms, enhancement of endogenous enzyme activity, stimulation of pancreatic secretions, stimulation of absorptive cell proliferation, and stimulation of intermediary metabolism. Even with all of these potential mechanisms, many studies have been anable to observe an improvement in nursery pig performance with the acidification of nursery pig diets. Possible dietary factors, which may influence the response to acidification include: type of acid, inclusion level, diet composition, dietary buffering capacity, age at weaning, length of inclusion post-weaning, and the existing level of performance in pigs fed a negative control diet.

Literature Review

Independent of weaning age, pigs undergo a post-weaning growth lag for approximately one week (Leibbrandt et al., 1975; Carroll et al., 1998). At weaning, the pig undergoes a transition from a highly palatable and digestible milk diet to a dry, cereal based diet. The delayed adaptation of the swine gastrointestinal tract to these more complex diets negatively impacts performance and may lead to gastric upsets such as diarrhea or scouring (Aherne et al., 1992). Major causes of digestive inefficiencies post-weaning include: insufficient production of certain carbohydrases (Chapple et al., 1989) and proteases (Makkink et al., 1994), and insufficient production and secretion of hydrochloric acid from the stomach (Cranwell, 1985; Doyle, 2001). Instabilities in gastric pH and ineffecient digestion can provide an optimal environment for the proliferation of pathogenic microorganisms in the gastrointestinal tract.

In an attempt to combat this post-weaning lag, numerous researchers have investigated the effects of acidying nursery pig diets with supplementary organic acids. Proposed modes of action of dietary acidification are discussed below:

Modes of Action of Dietary Acids

Gastic pH. Hydrochloric acid secreted by parietal cells in the gastric region of the stomach is responsible for the acidic conditions found in the stomach. Hydrochloric acid secretion is lowest at birth and increases with age (Cranwell, 1995). Chymosin, a milk clotting protease, is the dominant protease found in the stomach during the first 3-4 weeks of age. However, following weaning pepsinogen A increases and becomes the dominant protease. The pH optima for chymosin is between 3 and 5; whereas pepsinogen has a lower pH optima between 1 and 3. Therefore, while HCl secretion prior to weaning may be sufficient for chymosin activation and activity, it may be insufficient for pepsinogen activation and activity. Therefore, many researchers have attempted to reduce the gastric pH in nursery diets by acidifying the diet. The addition of organic acids to swine diets has been shown to decrease gastric pH. Radcliffe et al. (1998) fed weanling pigs citric acid at 1.5 and 3.0% inclusion levels. They observed a significant decrease in diet pH which caused a significant decrease in stomach pH at both levels of acid inclusion. However, Risley et al. (1992) fed citric and fumaric acid and did not see a change in gut pH at slaughter. Gastric pH changes with time and contents of the stomach, therefore, it is difficult to compare stomach pH between researchers unless the same slaughter procedures were carried out. Prior to slaughter, pigs should be fasted, fed a set amount, and killed a set time later to ensure all pigs being compared are at the same stage of digestion. Rice et al. (2002) observed a reduction in gastric pH in pigs fitted with an endogastric pH probe when diets containing 3.0% citric acid were fed.

Digesta transit time. As gastric pH decreases, gastric emptying slows. Therefore, there is additional time for digestion and absorption of nutrients throughout the gastrointestinal tract. As a result, it has been hypothesized that the acidification of nursery diets may increase gastric retention times and thereby increased nutrient absorption. However, very little direct evidence is available with regards to this mode of action.

Manipulation of gut microflora. There are two predominant mechanisms through which organic acids are hypothesized to alter microbial populations in the gastrointestinal tract. The first is simply a selection of "beneficial" commensal bacteria by a lowering of gastric and/or gastrointestinal pH. For instance, Fuller (1977) demonstrated that acidic conditions favor the growth of lactobacillus bacteria in the stomch, which may act to competititively exclude the growth of other potentially pathogenic bacteria. Furthermore, lactobacillus has the ability to destroy *E. Coli* through the production of lactic acid, which in turn lowers pH. Second, organic acids in their undisassoicated form may diffuse through semi-permeable bacterial membranes, and once inside disassociate in hydrogen ions and conjugate bases, thereby lowering cytosolic pH and inhibiting cytosolic enzyme activity (Lueck, 1980).

Pancreatic enzyme secretion. The secretory ability of the pancreas is not fully functional at the time of weaning. Pancreatic secretions are stimulated through a series of hormonal mechanisms under the control of the autonomic nervous system, Several researchers have observed increases in endocrine and exocrine pancreatic secretions in pigs as a result of increased intra-duodenal concentrations of short chain fatty acids (Harada et al., 1986; Sano et al., 1995).

Gut morphology. At weaning villous height decreases and crypt depth increases, resulting in a decreased absorptive capacity (Cera et al., 1988; Pluske et al., 1996). These alterations in morphology are likely a result of a reduction in feed intake immediately post-weaning. Little is known about the effects of diet acidification on gastrointestinal morphology. However, Sakata et al. (1988) reported that the production of short chain fatty acids from fermentation of fiber, resulted in an increased proliferation of epithelial cells lining the gastrointestinal tract. This should result in an increased absorptive capacity. In support of these findings, Galfi and Bokori (1990) observed an increase in micorvilli length and cell number with increasing concentrations of sodium butyrate in the ileal lumen.

Intermediary metabolism. It has been hypothesized that organic acids may stimulate intermediary metabolism resulting in improvements in energy

and/or protein/amino acid utilization (Kirchgessner and Roth, 1988). Studies carried out utilizing formic acid addition to weanling pig diets increased the activities of α-ketoglutaric dehydrogenase and glutamate-pyruvate transaminase (Grassmann et al., 1992). Studies were also carried out to investigate the effect of the addition of dietary fumaric acid on intermediary metabolism in the rat liver (Tschierschwitz et al., 1982; Grassmann and Klasna, 1986). Results from both of these studies showed no effects of fumaric acid on the activities of enzymes of the citric acid cycle. However, Tschierschwitz et al., (1982) observed increased activity of asparate tranferase and succinate dehydrogenase with the addition of fumaric acid to rat diets. Grassmann and Klasna (1986) noted that fumarase activity in the cytosolic fraction was increased by fumaric acid supplementation. They also found that liver transaminases, glutamate dehydrogenase, glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase activities were increased suggesting an increase in the synthesis of non-essential amino acids.

The Effects of Die Acidification on Nursery Pig Performance

Several studies (Falkowski and Aherne, 1984; Radcliffe et al., 1998; Boling et al., 2000) reported an improvement in growth performance of nursery pigs fed acidified diets. In a study by Jongbloed et al. (1996), an increased ADG was observed when growing pigs were fed diets containing lactic acid, formic acid, or propionic aid. Giesting et al. (1991) observed an improved growth performance in nursery pigs fed diets containing 3% formic acid. Krause et al. (1994) also observed an increase in growth performance of nursery pigs during first two weeks post-weaning, when fed fumaric acid supplemented diets. In studies by Falkowski and Aherne (1984), and Giesting and Easter (1991), an improvement in ADG of weaned pigs was observed when fed diets containing fumaric acid. Radcliffe et al. (1998) and Boling et al. (2000), observed a significant improvement in ADG when nursery pigs were fed diets containing citric acid. Diet acidification using organic acids can also improve feed efficiency in nursery pigs (Falkowski and Aherne, 1984; Boling et al., 2000). In their study, Kornegay et al. (1994) observed an improvement in body weight gain in weanling pigs fed acidified diets. In a study by Patience and Chaplin (1997) faster growth rates and a trend for improved feed efficiency was observed in pigs fed acidogenic diets (low dietary undetermined anion), compared to pigs fed control diets or partially compensated acidogenic diets (which had been supplemented with sodium and bicarbonate to elevate dietary undetermined anion).

The Effect of Diet Acidification on Nutrient Digestibility

Mroz et al. (2000) observed a tendency for improved ileal digestibility of DM, OM, and amino acids like arginine, isoleucine, phenylalanine, alanine, aspartic acid, and an increase in apparent total tract digestibilities of DM, ash, Ca, CP, and GE, when pigs were fed diets supplemented with calcium benzoate (used to obtain low buffering capacity). They observed a significant improvement in average calcium retention by 3.3% of its intake in growing pigs which were fed diets supplemented with calcium benzoate, compared to pigs fed diets containing calcium carbonate. However they observed a negative effect of calcium benzoate on P retention. Replacement of calcium carbonate with calcium benzoate decreased total P retention by 2.3 % units (expressed as percentage of intake). These researchers also observed an improvement in Ca and P retention when organic acids were supplemented to the diet. Babinszky et al. (1998) and Blank et al. (1999) observed a significant improvement in the ileal digestibility of crude protein and amino acids by up to 4.6 %-units with inclusion of 1% formic acid or 2% fumaric acid in cereal-soybean meal based diets for weanling pigs. Mosenthin et al. (1992) observed an improvement in the apparent ileal digestibilities of several indispensable amino acids when 2% propionic acid was supplemented to the diet. Mroz et al. (1998) reported an increase in apparent ileal digestibilities of CP and amino acids in growing pigs when supplementing formic, fumaric, or n-butyric acid at an inclusion rate of 300 mmol/kg to diets with varying buffering capacities. An improvement in nutrient digestibility was also observed in nursery pigs by Jensen et al. (1998), Radcliffe et al. (1998), and Partenen and Mroz (1999) when diets were supplemented with organic acids. However Waltz and Pallauf (1997) did not observe any improvement in amino acid digestibility or feed efficiency when piglets were fed diets supplemented with 1.5 % fumaric or citric acid.

Elevated gastric pH may lead to a decreased gastric proteolysis as a result of inefficient pepsin hydrolysis. As a result of this a greater proportion of

proteins may enter the small intestine intact, resulting in a decreased efficiency of protein digestion (Blank et al., 1999). In research by Makkink et al. (1994) an increased proteolysis was observed in early weaned pigs when fed skim milk powder, compared to pigs fed soybean meal. This indicates the inability of the young pig to properly utilize vegetable protein sources compared to animal protein (milk) sources after weaning. Studies in humans and dogs, by Mayer and Kelly (1976), and Mayer et al. (1976), showed that the end products of pepsin digestion namely peptides and amino acids, stimulate the secretion of pancreatic juice. Inadequate proteolysis in the stomach due to insufficient HCl secretion and resultant high gastric pH in young pigs might be a reason for low secretion of pancreatic enzymes (Linderman et al. 1986). Risley et al. (1992) measured the pH of digestive tract contents at 2 d pre-weaning, and 0, 3, 7, 14, and 21 d post-weaning in pigs fed diets supplemented with either fumaric or citric acids. They did not observe any reduction in gastric pH with acidification of starter diets. So, it is important to note that acidification of starter diets does not always lead to a reduction in gastric pH. One of the factors influencing the response of digestive tract pH to diet acidification could be dietary buffering capacity (Blank et al., 1999), and this may be one of the important reasons for the inconsistent results obtained in studies with organic acids on gastric pH.

According to Bolduan et al. (1998), buffering capacity of the diet is largely dependent on the source of protein and minerals in the diet. High buffering capacity diets resulting from supplementation of inorganic sources of calcium and (or) phosphorous, leads to lowered ileal digestibilities of OM and CP (Decuypere et al., 1997). Mroz et al. (1998) observed a non significant decrease in ileal digestibilities of CP and amino acids due to higher buffering capacity of the diet. Gabert et al. (1995a) did not detect differences in ileal digestibilities due to a higher buffering capacity of the diet. However, Blank et al. (1999) observed a good correlation (r^2= .44 to .68) between the apparent ileal digestibilities of amino acids on day 11 post-weaning and the concentration of fumaric acid in diets with low buffering capacity. However, no relationship between ileal digestibilities and fumaric acid supplementation to diets with a high buffering capacity was observed. So, an increased dietary buffering capacity has the potential to lower the digestibility of nutrients in young pigs.

According to Patience et al. (1987), dietary acidity or alkalinity can be manipulated using various inorganic mineral sources, which could serve as alternative source for pH modification in the diets of swine and poultry. According to Hardy (1992), of all the minerals Ca is the most active element affecting the acid-binding capacity or buffering capacity of diets. As stated by Petito and Evans (1984), sources of calcium and phosphorus can influence the acidity or alkalinity of the diet. Ca and P are the major minerals supplemented to monogastric diets. Since they have the capacity to alter the buffering capacity of the diet, careful selection of these mineral sources as buffering capacity modifiers, in place of other expensive acidifiers (organic acids and/or their salts) could be beneficial.

The Effects of Diet Acidification on Microbial Populations Colonizing the Gastrointestinal Tract

Much research has been carried out to evaluate the effects of organic acids on gut microflora yielding often variable results. These results have been primarily attributed to differences in the dietary acids used, their inclusion levels, pig health and composition of diets. A review by Mroz (2003), established the following order of killing potency of coliform bacteria for organic acids: propionic<formic<butyric <lactic<fumaric<benzoic. In another study, Jensen et al. (2001) demonstrated that the potency of organic acids against *Salmonella typhiumurium* in gastric digesta at pH 4 was in the following order: acetic< formic<propionic<lactic<sorbic<benzoic. Results from the literature indicate that dietary acid additions are only detected in the proximal GI tract and therefore the direct effect on microbial populations are expected in this segment (Jensen et al., 2003). A new micropackaging technique has being developed to produce a microcapsule which allows for the slow release of organic acids therefore allowing the entire GI tract access to the antimicrobial activity of the dietary acid. The encapsulation process also has the benefit of masking the acrid odors of some of acids, which will increase their palatability (Mroz, 2003).

Knarreborg et al, (2002) developed an in vitro method to study the effects of various dietary acids on the microbiota in the gastrointestinal tract of piglets. This tool allows for the evaluation of organic acids antimicrobial function and the subsequent identification of potential beneficial acids prior to in vivo

experimentation. Knarreborg et al. (2002) observed, using a batch culture system, that six different organic acids had the ability to reduce coliform and lactic acid bacteria in the stomach at pH 4.5. Lactic acid bacteria were noted to be more resistant to the action of organic acids than coliforms. Of the acids evaluated, benzoic acid had the most potent antimicrobial activity against coliforms followed by fumaric acid and these were the only acids which exhibited the ability to kill lactic acid bacteria. This study demonstrated a pH-dependent inhibition of bacterial growth; as the pH decreased the antibacterial activity of the organic acid increased when the concentration of the acid was held constant.

In vivo work with organic acids began as early as 1966 when Kershaw et al, reported a reduction in *E. coli* counts in the duodenum and jejunum of pigs receiving lactic acid in drinking water. Thomlinson (1981) also observed a reduction in the multiplication of *E. coli* 0141:K85 and consequently a reduction in piglet mortality as a result of acidification. Similar effects of organic acids on the coliform burden of the gastrointestinal tract have been observed (Bolduan et al, 1988b; Mattew et al, 1991; Johnson 1992) along with reductions in post-weaning scours (White et al, 1969).

Roth and Kirchgessner (1997) observed that formic acid addition at 1.2% for 6 to 12 kg pigs or 0.6% for 13 to 25 kg pigs reduced the frequency of post-weaning diarrhea. Formic acid did not alter gastrointestinal pH significantly however there was a reduction in the *E. coli* population of the duodenum and jejunum which explains the observed reduction in the incidence of diarrhea.

Lactic acid (0.7 to 2.8%) has been shown to increase the concentration of lactic acid bacteria and yeast along the gastrointestinal tract and decrease the abundance of coliforms (Maribo et al., 2000). The abundance of yeast is maintained under these circumstances as yeasts have the ability to metabolize lactic acid. Maribo et al, (2000b) proposed in order to maintain the lactic acid bacteria population and decrease populations of both yeast and coliforms a combination of formic acid and lactic acid should be used. There is also evidence that lactic acid (2.8%) has the ability to reduce the number of salmonella positive fecal samples from piglets (Jørgensen et al., 2002). Biagi et al. (2003) observed that the addition fumaric acid (1%) to a low buffering capacity diet resulted in lower counts of clostridia in the jejunum and lower counts of clostridia and coliforms in the cecum compared to the control. Counts of lactobacillus, eubacterium spp. and also the sum of the main flora in the duodenum, jejunum and ileum were significantly reduced as a result of fumaric acid inclusion at 1.8% in the diet of weanling pigs (Gedek et al., 1992b).

In contrast, some studies failed to observe any effect of organic acids on microbial populations. Risley et al., (1992) found no effect of fumaric acid (1.5%) on lactobacillus, coliforms or *E. coli* counts along the gastrointestinal tract and also observed that dietary acidification (1.5% fumaric or citric acid) did not prevent scouring and a growth lag from occurring following an *E. coli* challenge (Risley et al., 1993). Likewise, Clark and Batterman (1989) failed to detect any reduction in the incidence of scouring following dietary acidification.

Influence of Dietary Acids on Microbial Fermentation Patterns

Intestinal fermentation mainly occurs in the distal ileum and in the hindgut (Decuypere and Van der Heyde, 1972) where energy is the limiting factor for microbial growth. Such limitations may result in increased proteolysis and release of toxic substances, such as ammonia and amines (Ørskov et al., 1970; Russell et al., 1983). Amino acid degradation by small intestinal bacteria deprives the pig of nutrients and the toxic catabolites produced have the potential to affect both the nutritional and physiological status of the animal primarily by interference with the growth and differentiation of intestinal epithelial cells (Gaskins, 2001).

One of the amino acid catabolites that bacteria can produce are amines. They are formed via decarboxylation of amino acids and polyamines. The most common amines produced which are pharmacologically active in the gut include cadaverine, histamine, putrescine and tyramine (Drasar and Hill, 1974). Increased amine production by intestinal bacteria has been associated with diarrhea at weaning in pigs (Porter and Kenworthy, 1969).

Another toxic catabolite of microbial amino acid degradation and urea hydrolysis is ammonia. A wide range of intestinal bacteria exhibit urease and therefore have the ability to convert urea which is synthesized by the liver to ammonia. The toxic ammonia is absorbed by the colon and normally undergoes detoxification via reconversion to urea in the liver. However, ammonia concentrations in the colon of a

conventional pig are commonly several times greater than the capacity of the liver for detoxification. There is evidence to suggest that high ammonia concentrations arising from both amino acid deamination and urea hydrolysis can depress growth performance of pigs (Visek, 1978a). Ammonia increases epithelial cell turnover by effecting cellular metabolism and DNA synthesis (Visek, 1972, 1978b). Urea hydrolysis does not occur in germfree animals (Levenson et al., 1959) and epithelial cell turnover is also substantially reduced (Galjaard et al., 1972). Visek et al, (1978a) has hypothesized that the reduction in microbial produced ammonia is the primary mechanism by which antibiotics carry out their function. Microbial produced ammonia can also be reduced through the addition of ion-exchange resins capable of ammonia absorption (Pond and Yen, 1987). Also the addition of a fermentable carbohydrate source which enables the bacteria to use ammonia as a nitrogen source for the synthesis of microbial protein reduces the ammonia burden on epithelial cells (Byrant, 1974).

Ammonia content in the stomach was lowered by increasing dosage of formic acid (0.6 -2.4%) but was not significantly affected in other parts of the gastrointestinal tract (Eckel et al., 1992b). In the small intestine, concentrations of amines (cadaverine, putrescine and spemidine) were not affected by formic acid supplementation. The addition of formic acid at 1.25% resulted in significantly lower levels of ammonia in the stomach and small intestine however the addition of the buffer, sodium hydroxide, increased ammonia production as a consequence of creating a more favorable environment for bacterial growth (Eidelsburger et al., 1992a). Monsenthin et al, (1992) observed that the addition of propionic acid at 2% had no effect on the concentrations of ammonia or amines in ileal digesta.

Effects of Diet Acidification on Fecal and Urinary pH

Livestock waste is a potential source of environmental pollution and its associated problems. Ammonia is one of the major environmental pollutants originating from the livestock industry, resulting from inadequate utilization of feed nitrogen (i.e., feed protein). Inefficient protein utilization leads to excretion of nitrogen in the form of urea via the urine. Urea is converted to ammonia by the catalytic action of urease. Improving feed nutrient utilization and minimizing urinary nitrogen excretion, results in a reduction of ammonia emission into the atmosphere. Another important strategy suggested by several researchers (Cahn et al. 1997; Sutton et al. 1997; Mroz et al 2000a) to reduce ammonia volatilization into the atmosphere is manipulation of manure or urinary pH by diet modification. In a study by Cahn et al. (1997), a lowered urine pH (ranged from 1.6 to 1.8 units) and slurry pH was achieved by decreasing the dietary buffering capacity (achieved by replacing dietary calcium carbonate which is alkalogenic by acidogenic calcium salts, such as calcium sulphate). These researchers also observed a reduction in ammonia emissions by 26% to 53% by including acidogenic Ca salts up to dietary Ca concentrations of 7g/kg to 10g/kg. Sutton et al. (1997), observed a reduction in swine manure pH when 5% cellulose was added to the diet. Hendricks et al. (1997) observed a 37% reduction in ammonia emissions by feeding calcium benzoate to grow-finishing pigs in place of calcium carbonate. Kim and van Kempen (2001) observed a reduction in ammonia emissions associated with growing pigs, by 30%, when fed diets containing a combination of phosphoric acid and calcium sulphate, and by 15% when fed diets contained a combination of monocalcium phosphate and calcium sulphate. From these results, it can be concluded that diet manipulation of acidogenic agents can reduce ammonia emission from swine manure. In their study Mroz et al. (2000b) observed a reduction in urine pH in pigs fed diets containing acidogenic calcium sulphate, when compared to pigs fed diets containing alkalogeinc calcium carbonate. In another study by Mroz et al. (2000a) a lowered urine pH was observed when pigs were fed Ca sulphate compared to calcium carbonate.

The Role of Dietary Buffering Capacity

It is of utmost importance that the gastric pH of newly weaned pigs remains low in order to initiate protein digestion and to create an inhospitable environment for the colonization of pathogenic bacteria. Some feed has the ability to bind more acid than others (Jasaitis et al., 1987; Bolduan et al., 1988a,b) and the use of such feeds in starter diets will help to maintain a higher gastrointestinal pH. It is therefore possible to manipulate diet buffering capacity through the careful selection of ingredients that do not resist changes in pH (Jasaitis et al., 1987; Bolduan et al., 1988a,b).

There are a number of ways in which the acid buffering capacity of feed can be determined. Jasaitis et al. (1987) defined the titration value (TV) of

feed as the amount of HCl in milliequivalents (meq) required to lower 0.5 g of a feed sample to pH 4. The acid buffering capacity of the feed which was defined as the amount of acid required to cause a one unit pH change was calculated by dividing the TV by the change in pH of the feed from its initial value to 4. Straw et al. (1991) determined the TV of 10 g of digesta by mixing it with 50ml of deionized water overnight and then adding 0.2 N HCl until pH 2 was reached. The sample was then back-titrated to pH 8 using 0.2 N NaOH. The TV was calculated by subtracting the meq of NaOH/kg of digesta required to increase pH from 2 to 8 from the meq of HCl/kg digesta required to lower the pH 2. Bolduan et al, (1988) defined the acid binding capacity of feed as the millimoles of HCl required to reduce the pH of a 100g of sample to pH 4.

Factors Effecting Buffering Capacity of Feed

Feed type. The acid buffering capacity of different feeds varies substantially. Cereals and cereal by-products are known to have the lowest buffering capacity, protein feedstuffs have intermediate to high buffering capacity and mineral sources have the highest buffering capacity with the exception of monosodium phosphate and dicalcium phosphate (Partanen and Mroz, 1999). Roth and Kirchgessner, (1989a) found that the addition of mineral sources and protein to diets weakened the pH lowering ability of acids. A study conducted by Kornegay et al. (1994) observed that when pigs were fed a 16% CP diet, bone stress values increased as diets became less acidic, however stress values were unaffected by diet acidity when 22% CP diets were fed. This suggests that protein acts as a buffer to modify the acidic potential. Consequently, its seems plausible that feed ingredient selection greatly influences dietary buffering capacity and may help to explain some of the variability experienced in relation to the efficacy of dietary acidifiers.

Mineral sources. The mineral content of feed is an important factor influencing its ability to bind acid (Jasaitis et al., 1987; Bolduan et al., 1988). The ratio of cations to anions in feed ingredients remains constant even though individual concentrations of each may fluctuate (Jasaitis et al., 1987). Lawlor (1992) found that the dietary buffering capacity of a variety of feedstuffs was correlated ($P<.001$) with cation-anion difference [(Ca + Mg + K + Na) – (Cl + N + P + Si + S) and (Ca + Mg + K + Na) – (Cl + P + S)], total cations (Ca + Mg + K + Na) and total ash. Mineral sources such as calcium carbonate and defluorinated phosphate are more alkaline minerals and have high buffering capacity compared to dicalcium phosphate and calcium sulfate which are more acidic and have low buffering capacity (Straw et al., 1991; Kornegay et al., 1994).

Influence of Buffering Capacity on the Effectiveness of Dietary Acidification

The choice of mineral source can have a substantial influence on acid buffering capacity of the diet together with the amount of mineral content (Roth and Kirchgessner, 1989a). Blank et al. (1999) observed that a high buffering capacity diet brought about by supplementation of 3% sodium bicarbonate decreases apparent ileal digestabilities of CP and amino acids. Likewise Decuypere et al, (1997) showed that a high buffering capacity diet as a result of supplementation with inorganic calcium and (or) phosphorus sources, leads to lower ileal digestibilities of OM and CP. In contrast, Gabert et al. (1995) did not detect differences due to high buffering capacity. Kornegay et al. (1994) reported an improvement in growth rate as a result of manipulation of calcium and phosphorus sources to yield diets with low buffering capacity. They also reported failure to detect a lowering of gastric or intestinal pH as a result of diet acidification which concurs with findings of other researchers (Straw et al., 1991; Risley et al., 1991, 1992).

The response to acidification of starter diets reveals a considerable variation in the action of different organic acids. One possible explanation for these inconsistencies is the difference in acid buffering capacity of the diets. Blank et al. (1999) reported a positive correlation between ileal digestibilities of GE and CP and fumaric acid supplementation to the diets with low buffering capacities. This relationship was absent when fumaric acid was supplemented to diets with high buffering capacity. However, the positive effects of fumaric acid supplementation and low buffering capacity were only observed during the first 3 to 4 weeks post-weaning. This suggests that the beneficial effects of fumaric acid may be related to morphological changes in the young pig and further maturation of its own digestive system makes organic acid supplementation redundant.

In contrast, Gabert et al. (1995) concluded that the addition of formic acid to diets with high and low buffering capacity did not affect the apparent ileal

digestabilities of AA. The study also reported that there was no effect on nutrient digestibility or microbial populations in the small intestine. The findings from a study conducted by Biagi et al. (2003) however state that reducing the buffering capacity of the diet positively influenced the composition of intestinal microflora, reducing clostridia in the jejunum and clostridia and coliforms in the caecum. They concluded that feeding low buffering capacity diets in conjunction with citric or fumaric acid can result in growth rates similar to that of plasma protein diets and enable the population of undesirable bacteria to be kept in check.

Conclusions

Diet acidification has been shown to improve weanling pig performance. Known and proposed modes of action include: reductions or stabilization of gastric pH, alterations in gut microflora populations through bacteriostatic or bactericidal mechanisms, enhancement of endogenous enzyme activity, stimulation of pancreatic secretions, stimulation of absorptive cell proliferation, and stimulation of intermediary metabolism. However, response to diet acidification may be affected by: type of acid, inclusion level, diet composition, dietary buffering capacity, age at weaning, length of inclusion post-weaning, and the existing level of performance in pigs fed a negative control diet. Recent research at Purdue University has demonstrated that there may be beneficial effects to altering the type of acid included in nursery diets (i.e. rotational schemes with inorganic and organic acid blends). Water acidification has also been shown to be effective. However, recent work at Purdue has also demonstrated that you can provide "too much of a good thing". Combining water acidification with diet acidification resulted in a depression in feed intake and subsequent performance.

Future research is needed to further delineate proper rotational strategies with dietary acidifiers, and the role of dietary buffering capacity on acidifier effectiveness. Lalitha et al. (2003) demonstrated a reduction in gastrointestinal pH in broilers fed a low buffering capacity diet compared to broilers fed a control diet. Therefore, the level of acid needed to elicit a response may differ drastically depending on the buffering capacity of the diet.

References

Aherne, F., M. G. Hogberg, E. T. Kornegay, G. C. Shurson, M. Brocksmith, S. Broksmith, G. R. Hollis and J. E. Pettigrew. 1992. Management and nutrition of the newly weaned pig. PIH 111. National Pork Producers Handbook.

Babinszky, L., J. Tosenberger, L. Polmai, P. Karakas, J. Szabo, and P. Horn. 1998. The effects of formic acid on ileal digestibility of amino acids and on performance in weaned piglets. J. Anim. Sci 76(Suppl. 1):181(Abstr).

Biagi, G., A. Piva, T. Hill, D. K. Schneider, and T. D. Crenshaw. 2003. Low buffering capacity diets with added organic acids as a substitute for antibiotics in diets for weaned pigs. In: Proceedings of the 9th International Seminar on Digestive Physiology in the Pig. 2: 217-219.

Blank, R., R. Mosenthin, W. C. Sauer, and S. Huang. 1999. Effects of fumaric acid and dietary buffering capacity on ileal and fecal amino acid digestibilities in early-weaned pigs. Journal of Animal Science. 77: 2974-2984.

Bolduan, G., H. Jung, R. Schneider, J. Block, and B. Klenke. 1988. Recent advances in the nutrition of weaner pigs. Pig News Info. 9:381-385.

Bolduan, G., H. Jung, R. Schneider, J. Block, and B. Klenke. 1988b. Influence of fumaric acid and propanediol formate on piglets. Journal of Animal Physiology and Animal Nutrition. 59: 143-149.

Bolduan, G., H. Jung, R. Schneider, J. Block, and B. Klenke. 1988a. Influence of propionic and formic acids on piglets. Journal of Animal Physiology and Animal Nutrition. 59: 72-78.

Boling, S. D., D. M. Webel, I. Mavromichalis, C. M. Parsons, and D. H. Baker. 2000. The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. Journal of Animal Science 78: 682-689.

Byrant, M. P. 1974. Nutritional features and ecology of predominat anaerobic bacteria of the intestinal tract. American Journal of Clinical Nutrition. 27: 1313.

Cahn, T. T., A.J.A. Aarninck, Z. Mroz, and A. W. Jongbloed. 1997. Influence of dietary calcium salts and electrolyte balance on urinary pH, slurry pH and ammonia volatilization from slurry of growing finishing pigs. J. Anim. Sci. 75 (suppl 1):1989.

Carroll, J.A., Veum, T. L. and R. L. Matteri. 1998. Domestic responses to weaning and changes in post-weaning diet in the young pig. Domestic Anim. Endocri. 15:183-194.

Cera, K. R., D. C. Mahan, R. F. Cross, G. A. Reinhart, and R. E. Whitmoyer. 1988. Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. Journal of Animal Science. 66: 574-584.

Chapple, R. P., J. A. Cuaron, and R. A. Easter. 1989. Effect of glucocorticoids and limiting nursing on the carbohydrate digestive capacity and growth rate of piglets. J. Anim. Sci. 67:2956–2973.

Clark, W. A., and E. S. Batterham. 1989. Citric acid supplementation of creep-weaner diets. In: J. L. Barnett, and D. P. Hennessy (eds) Manipulating pig production II. pp. 137. Werribee, Victoria: Australasian Pig Science Association.

Cranwell, P. D. 1985a. The development of the stomach in the pig: The effects of age and weaning: 1. Stomach size, muscle and zones of mucosa. In: Proceedings of the 3rd International Seminar on digestive physiology in the pig. Report. No. 580: 112-115.

Cranwell, P. D. 1995. Development of the neonatal gut and enzyme systems. In: the Neonatal Pig, Development and Survival.

Decuypere, J. A., and H. Van der Heyde. 1972. Study of the gastrointestinal microflora of suckling piglets and early weaned piglets reared using different feeding systems. Zentralbl. Bakteriol. Parasitenkd. Infektionsk. Hyg. I A221: 492-510.

Decuypere, J., M. De Bruyn, and N. Dierick. 1997. Influence of the buffering capacity of the feed on the precaecal digestibility in pigs. In: Proceedings of the IIVth International Symposium on Digestive Physiology in Pigs. EAAP Publ. No. 88 :391-394. Saint Malo, France.

Doyle. M. E. 2001. Alternatives to antibiotic use for growth promotion in animal husbandry. FRI Briefings.

Drasar, B. S., and M. J. Hill. 1974. Human Intestinal Flora, Acedemic Press, London.

Eckel, B., F. X. Roth, M. Kirchgessner, and U. Eidelsburger. (1992b). Influence of formic acid on concentrations of ammonia and biogenic amines in the gastrointestinal tract. 4. Nutritive value of organic acids in piglet rearing. Journal of Animal Physiology and Animal Nutrition. 67:198-205.

Eidelsburger, U., M. Kirchgessner, and F. X. Roth. 1992a. Influence of formic acid, calcium formate and sodium bicarbonate on pH, concentration of carbonic acids and ammonia in different segments of the gastrointestinal tract. 8. Nutritive value of organic acids in piglet rearing. Journal of Animal Physiology and Animal Nutrition. 68:20-32.

Falkowski, J. F., and F. X. Aherne. 1984. Fumaric and citric acid as feed additives in starter pig nutrition. Journal of Animal Science. 58: 935-938.

Fuller, R. 1977. The importance of lactobacilli in maintaining normal microbial balance in the crop. British Poultry Science. 18: 8-94.

Gabert, V. M., W. C. Sauer, M. Schmitz, F. Ahrens, and R. Mosenthin. 1995a. The effect of formic acid and buffering capacity on the ileal digestibilities of amino acids and bacterial populations and metabolites in the small intestine of weanling pigs fed semipurified fish meal diets. Canadian Journal of Animal Science. 75: 615-623.

Galfi, P., and J. Bokori. 1990. Feeding trial in pigs with a diet containing sodium n-butyrate. Acta Veterinaria Hungarica. 38: 3-17.

Galjaard, H., W. van ger Meer-Fieggen, and J. Giesen. 1972. Feedback control by functional villus cells on cell proliferation and maturation in intestinal epithelium. Experimental Cell Research. 73:197.

Gaskins, H. R. 2001. Intestinal bacteria and their influence on swine growth. In: Lewis, A. J, L. L. Southern (eds) Swine Nutrition. 585-608. CRC Press, Boca Raton, FL.

Gedek, B., F. X. Roth, M. Kirchgessner, S. Wiehler, A. Bott, and U. Eidelsburger. 1992b. Influence of fumaric acid, hydrochloric acid, sodium formate, tylosin, and toyocerin on the microflora in different segments of the gastrointestinal tract. 14. Nutritive value of organic acids in piglet rearing. Journal of Animal Physiology and Animal Nutrition. 68: 1288-1294.

Giesting, D. W., M. A. Roos, and R. A. Easter. 1991. Evaluation of the effect of fumaric acid and sodium bicarbonate addition on performance of starter pigs fed diets of different types. Journal of Animal Science. 69: 2489-2496.

Grassmann, E. and T. Klasna. 1986. Comparison studies on the effect of supplements of fumaric and citric acids on body composition and enzyme activities of rats differently supplied with portein. Landwirtschaftliche Forschung. 39: 307-319.

Grassmann, E., F. X. Roth, and M. Kirchgessner. 1992. Metabolic effects of formic acid in daily use. 6. Nutritive value of organic acids in piglet rearing. Journal of Animal Physiology and Animal Nutrition. 67: 250-257.

Harada, E., M. Niiyama, and B. Syuto. 1986. Comparison of pancreatic exocrine secretion via endogenous secretin by intestinal infusion of hydrochloric acid and monocarboxylic acid in anesthetized piglets. Japanese Journal of Physiology. 36: 843-858.

Hardy, B. 1992. Diets for young pig. In: Neonatal Survival and Growth. Pp 99-107. (Eds. M. A. Varley, P. E. V. Williams, and T. L. J. Lawrence. Occational publication No.15. British Society of Animal Production.

Hendricks, G.L., M. G. M. Vrielink, and C.M.C. van der Peet-Schwering. 1997. Reducing ammonia emission from pig housing by adding acid salts to the feed. Pages 65-70. In Proceedings of the Fifth International Livestock Environment Symposium, ASAE, St. Joseph, MI.

Jasaitis, D. K., J. E. Wohlt, and J. L. Evans. 1987. Influence of feed ion content on buffering capacity of ruminant feedstuffs in vitro. Journal of Dairy Science. 70: 1391-1403.

Jensen, B. B. 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. J Anim Feed Sci. 7(Suppl 1):45–64.

Jensen, B. B., L. L. Maikkelsen, N. Canibe, and O. Høyberg. 2001. Salmonella in slaughter pigs. Annual Report 2001 from the Danish Institute of Agricultural Science Research Centre Foulum, Ttjele, Denmark.p. 23.

Jensen, B. B., O. Høyberg, L. L. Maikkelsen, M. S. Hedemann, and N. Canibe. 2003. Enhancing intestinal function to treat and prevent intestinal disease. In: R. O. Ball (ed) Proceeding of the 9th International Symposium on Digestive Physiology in Pigs, Banff, AB, Canada. 1: 103-119.

Johnson, R. 1992. Role for acidifiers and enzymes in assuring performance and health of pigs post-weaning. In: Biotechnology in the Feed Industry (Proc. Alltech's Eight Annual Symp.). 149-150. Alltech Technical Publications, Nicholasville, KY, USA.

Jongbloed, A.W.., P. A. Kemme, Z. Mroz, and R. Jongbloed. 1996. The effect of organic acids in diets for growing pigs on the efficacy of microbial phytase. Pp 515-524.In: Animal nutrition and waste management. M. B. Coelho, AND e. t. Kornegay (Eds.). BASF Reference Manual.

Jørgensen, L., C. F. Hansen, K. E. Bach Knudsen, B. B. Jensen, and H. Kjærsgaard. 2002. Particle size in meal feed to slaughter pigs. Effect on productivity, Salmonella prevalence and the gastrointestinal ecosystem (in Danish). Publication no. (in press). The National Committee for Pig Production, Copenhagen, Denmark.

Kim, I., B. and T. van Kempen. 2001. Determination of ammonia emission and urine pH as affected by different dietary sources of calcium and/or phosphorus in gro-finish pigs. Annual swine report, 2001. North Carolina State University.

Kirchgessner, V. M., and F. X. Roth. 1988. Energy value of organic acids in the rearing of piglets and the fattening of pigs. Ubersichten zur Tierernahrung.16: 93-108.

Knarreborg, A., N. Miquel, T. Granli, and B. B. Jensen. 2002. Establishment and application of an in vitro methodology to study the effects of organic acids on coliform and lactic acid bacteria in the proximal part of the gastrointesinal tract of piglets. Animal Feed Science and Technology. 99: 131-140.

Kornegay, E. T., J. L. Evans, and V. Ravindran. 1994. Effects of diet acidity and protein concentration or source of calcium on the performance, gastrointestinal content measurements, bone measurements, and carcass composition of gilt and barrow weanling pigs. J. Anim. Sci. 72:2670-2680

Kornegay, E. T., J. L. Evans, and V. Ravindran. 1994. Effects of diet acidity and protein level or source of calcium on the performance, gasstrointestinal content measurements, bone measurements and carcass composition of gilt and barrow weanling pigs. Journal of Animal Science. 72: 2670-2680

Krause, D. O., P. C. Harrison, and R. A. Easter. 1994. Characterization of the nutritional interactions between organic acids and inorganic bases in the pig and chick. Journal of Animal Science. 72: 1257-1262.

Lawlor, P. G.1992. Studies on the use of fumaric acid in diets for weanling pigs. PhD thesis. University College Dublin, Ireland.

Leibbrandt, V. D., R. C. Ewan, V. C. Speer, and D. R. Zimmerman. 1975. Effect of weaning and age at weaning on baby pig performance. J. Anim. Sci. 40:1077-1080.

Levenson, S. M., L. V. Cromley, R. E. Horowitz, and O. J. Malm. 1959. The metabolism of carbon-la-

beled urea in the germfree rat. Journal of Biological Chemistry. 234:2061.

Lindemann, M. D., S. G. Cornelius, S. M. El Kandelgy, R. L. Moser, and J. E. Pettigrew. 1986. Effect of age, weaning and diet on digestive enzyme levels in the piglet. Journal of Animal Science. 62: 1298-1307.

Lueck, E. 1980. Antimicrobial food additives: Characteristics, uses, effects. Springer-verlag, Berlin, Germany.

Makkink, C. A., G. P. Negulescu, Q. Guixin, and M. W. A. Vertegen. 1994. Effects of dietary protein source on feed intake, growth, prancreatic enzyme activities and jejunal morphology in newly weaned piglets. British Journal of Nutrition. 72: 353.

Maribo, H., L. E. Olsen, B. B. Jensen, and N. Miquel. 2000. Different doses of organic acids to piglets. Danish Bacon and Meat Council, no. 469 (In Danish).

Maribo, H., L. E. Olsen, B. B. Jensen, and N. Miquel. 2000b. Combination of lactic acid and formic acid and benzoic acid to piglets. Publication no. 490. The National Committee for Pig Production, Copenhagen, Denmark.

Matthew, A. G., A. L. Sutton, A. B. Scheidt, D. M. Forsyth, J. A. Patterson, and D. T. Kelly. 1991. Effects of a propionic acid containing feed additive on performance and intestinal microbial fermentation of the weanling pig. In: Proceedings of the 5th International Seminar on digestive physiology in the pig. EAAP Publication No 54: 464-469.

Mayer, J. H., and G. A. Kelly, and R. S. Jones. 1976. Canine pancreatic response to intestinally perfused oligopeptides. Am. J. Physiol. 231:678-681.

Mayer, J. H., and G. A. Kelly. 1976. Canine pancreatic responses to intestinally perfused proteins and protein digests. Am. J. Physiol. 231:682-691.

Monsenthin, R., W. C. Sauer, F. Ahrens, C. F. M. de Lange, and U. Bornholdt. 1992. Effects of dietary supplements of propionic acid, siliceous earth or a combination of these on the energy, protein and amino acid digestibilities and concentrations of microbial metabolites in the digestive tract of growing pigs. Animal Feed Science and Technology. 37: 245-255.

Mroz, Z. 2003. Organic Acids of various origin and physico-chemical forms as potential alternatives to antibiotic growth promoters for pigs. In: Proceeding of the 9th International Symposium on Digestive Physiology in Pigs. 1: 267-293. Banff, AB, Canada.

Mroz, Z., A. J. Moeser, K. Vreman, J. T. van Diepen, T. van Kempen, T. T. Canh, and A. W. Jongbloed. 2000a. Effects of dietary carbohydrates and buffering capacity on nutrient digestibility and manure characteristics in finishing pigs. J. Anim Sci. 78: 3096-3106.

Mroz, Z., A. W. Jongbloed, K. H. Partanen, J. T. M. van Diepen, K. Vreman, and J. Kogut. 1998. Ileal digestibility of amino acids in pigs fed diets of different buffering capacity and with supplementary organic acids. J. Anim. Feed. Sci. 7(Suppl. 1):191-197.

Mroz, Z., A. W. Jongbloed, K. H. Partanen, K. Vreman, P. A. Kemme, and J. Kogut. 2000. The effects of calcium benzoate in diets with or without organic acids on buffering capacity, apparent digestibility, retention of nutrients, and manure characteristics in swine. Journal of Animal Science. 78: 2622-2632.

Mroz, Z., A.W. Jongbloed, K. H. Partanen, *K. Vreman, P. A. Kemme, and J. Kogut.* 2000b. The effects of calcium benzoate in diets with or without organic acids on dietary buffering capacity, apparent digestibility, retention of nutrients, and manure characteristics in swine. J. Anim Sci. 78: 2622-2632.

Ørskov, E. R., C. Fraser, V. C. Mason, and S. O. Mann. 1970. Influence of starch digestion in the large intestine of sheep on cecal fermentation, cecal microflora and fecal nitrogen excretion. British Journal of Nutrition. 24: 671-682.

Partanen, K. H., and Z. Mroz. 1999. Organic acids for performance enhancement in pig diets. Nutrition Research Reviews. 12: 117-145.

Patience, J. F., and R. K. Chaplin. 1997. The Relationship Among Dietary Undetermined Anion, Acid-Base Balance, and Nutrient Metabolism in Swine. J. Anim. Sci. 75:2445–2452.

Patience, J. F., R. E. Austic, and R. D. Boyd. 1987. Effect of dietary electrolyte balance on growth and acid-base balance. Journal of Animal Science. 64: 457-466.

Petito, S. L., and J. L. Evans. 1984. Calcium status of the growing rat as affected by diet acidity from ammonium chloride, phosphate and protein. Journal of Nutrition. 114: 1049-1059.

Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Journal of Animal Science. 62: 131-144.

Pond, W. G., and J. T. Yen. 1987. Effect of carbadox, an antibiotic combination or clinoptilolite on

weight gain and organ weights of growing swine fed maize or rye as the grain sources. Nutrition Report International. 35:801.

Porter, P., and R. Kentworthy. 1969. A study of intestinal and urinary amines in pigs in relation to weaning. Research in Veterinary Science. 10:440.

Radcliffe, J. S., Z. Zhang, and E. T. Kornegay. 1998. The effects of microbial phytase, citric acid and their interactions in a corn-soybean meal-based diet for weanling pigs. Journal of Animal Science. 76: 1880-1886.

Risley, C. R., E. T. Kornegay, M. D. Lindemann, C. M. Wood, and W. N. Eigel. 1993. Effect of feeding organic acids on gastrointestunal digesta measurements at various times postweaning in pigs challanged with enterotoxigenic *E. Coli.* Canadian Journal of Animal Science. 73: 931-940.

Risley, C. R., E. T. Kornegay, M. D. Lindemann, C. M.Wood, and W. N. Eigel. 1992. Effects of feeding organic acids on selected intestinal content measurements at varying times postweaning in pigs. Journal of Animal Science. 70: 196-206.

Roth, F. X., and M. Kirchgessner. 1989a. Significance of dietary pH value and buffer capacity in piglet feeding. 2. Effect of pH lowering in the feed by inclusion of ortho-phosphate on growth and feed conversion. Landwirtschaft Forschung. 42: 168-175.

Roth, F. X., and M. Kirchgessner. 1997. Formic acid as a feed additive for piglets: Nutritional and gastrointestinal effects. In: J. P. Laplace, C. Fevrier, and A. Barbeau (eds) Proceeding of the 7th International Symposium on Digestive Physiology in Pigs, Saint Malo, France. pp. 498-501.

Russell, J. B., C. J. Sniffen, and P. J. Van Soest. 1983. Effects of carbohydrate limitation on degradation and utilization of caesin by mixed rumen bacteria. Journal of Dairy Science. 66: 763-775.

Sakata, T. 1988. Chemical and physical trophic effects on dietary fiber on the intestine of monogastric animals. In: L. Buraczewska, S. Buraczewski, B. Pastuszewska, and T. Zebrowska (Eds) Digestive physiology in the pig.128-135. Polish Academy of Science, Jablonna.

Sano, H., E. H. Akamaura, H. Takahashi, and Y. Terashima. 1995. Plasma insulin and glucagon responses to acute challenges of acetate, propionate, n-butyrate and glucose in growing gilts (sus scrofa). Comparative Biochemistry and Physiology 110A: 375-378.

Straw, M. L., E. T. Kornegay, J. L.Evans, and C. M. Wood. 1991. Effects of dietary pH and phosphorus source on performance, gastrointestinal tract digesta, and bone measurements of weanling pigs. Journal of Animal Science. 69: 4496-4504.

Sutton, A.J., K.B. Kephart, J. A. Patterson, R. Mumma, D. T. Kelly, E. Bogus, B. S. Don, D. D. Jones, and A. J. Heber. 1997. Dietary manipulation to reduce ammonia and odorous compounds in excreta and anaerobic manure storage. Pages 245-252. In: International Symposium on Ammonia and Odour Control from Animal Production Facilities, Rosmalen, The Netherlands.

Thomlinson, J. R. 1981. Dietary manipulation of gastric pH in the prophylaxis of enteric disease in weaned pigs: Some field observations. Veterinary Record. 109: 120-122.

Tschierschwitz, A., E. Grassmann, M. Kirchgessner, and F. X. Roth. 1982. The effect of fumaric acid supplements of activities of liver enzymes (GOT, GPT, SUCCDH) with different supplies of energy and protein to growing rats. Zeitschrift fur Tierphysiologie, Tierernahrung und Futtermittelkunde. 48: 253-259.

Visek, W. J. 1972. Effects of urea hydrolysis on cell life-span and metabolism. American Journal of Clinical Nutrition. 31:S216.

Visek, W. J. 1978a. The mode of growth promotion by antibiotics. Journal of Animal Science. 46: 1447-1469.

Visek, W. J. 1978b. Diet and cell growth modulation by ammonia. Fed. Proc. 30: 1760.

Walz O. P. and J. Pallauf. 1997. Retention and utilization of amino acids in piglets fed ad libitum or restrictively diets supplemented with organic acids. Arch Anim Nutr. 50(3):227–238.

White, F., G. Wenham, G. A. M. Sharman, A. S. Jones, E. A. S. Rattray, and I. McDonald. 1969. Stomach function in relation to stomach scouring in the pig. British Journal of Nutrition. 23: 847-857.

High Quality Protein Sources for Young Pigs

Tip Cline and Ryan Dilger
Department of Animal Sciences
Purdue University
915 West State Street
West Lafayette, IN 47907-2054
765-494-4846
tcline@purdue.edu

Summary

Considerable research published in the scientific literature shows conclusively that spray dried animal plasma (SDAP) added to phase 1 starter diets will enhance gain and feed intake of pigs. The first week post-weaning is usually when the biggest response is observed. Spray dried porcine plasma (SDPP) is superior to spray dried bovine plasma. The immunoglobulin fraction appears to be responsible for a major portion of the growth response. Many potential replacements for SDAP have been used in diets for early weaned pigs including fish meal, milk proteins, egg proteins, soy proteins and wheat gluten, but none have proven equal to plasma products. It is reasonable to assume that the amino acid pattern of SDAP can be duplicated, but the apparent benefit of passive immunity received by the pig from the globular portion of the product has not been duplicated. Bioengineering of milk producing animals, laying hens and/or microorganisms to produce the beneficial globular proteins of SDAP may be possible in the future.

Introduction

Newly weaned pigs may perform poorly if not fed a high quality diet that contains relatively high levels of animal products. These pigs if fed a fortified corn-soy diet may actually lose weight for a few days and may develop diarrhea, which further compounds the feed intake and growth "lag." It is well known that diets containing relatively high levels of lactose and animal proteins support higher performance levels than "vegetarian" diets. Accepted also is that the inclusion of spray dried plasma protein in phase 1 diets is essential to maximize rate of gain for the first couple of weeks after weaning. A question worthy of consideration today is, are there replacement strategies available if the pig industry could not use any animal by-product in feed?

Spray Dried Animal Plasma

Several studies have been conducted with SDAP since the early work at Iowa State (Gatnau and Zimmerman, 1990) that showed improved performance of pigs fed SDAP in the starter diet. Hansen et al. (1993) showed conclusively that starter pigs fed spray dried porcine plasma consumed more feed and gained more rapidly than pigs fed spray dried blood or spray dried bovine plasma. The blood products replaced dried skim milk (DSM) or dried whey in their studies.

Attempts have been made to explain the reason(s) for SDAP's superiority in phase 1 starter diets and several possible modes of action have been put forth. Ermer et al. (1994) suggested that SDPP is more palatable than a combination of dried why, dried skim milk and lactose. They showed conclusively that pigs prefer a diet containing SDPP as compared to dried skim milk, but that does not prove that "palatability," per se, explains performance differences.

Considerable evidence has been accumulated indicating that the immunoglobulin (IgG) fraction of SDAP plays a major role in performance enhancement. Several abstracts were published in 1995 (Weaver et al.; Pierce et al.; Owen et al.; Gatnau et al.) showing that most of the performance response due to feeding SDPP in the starter diet was due to

the IgG fraction. The response obtained from the albumen fraction was usually positive, but variable, whereas the response from a low molecular weight fraction was often negative. Coffey and Cromwell (1995) suggested that the response was probably due to improved immunocompetence gained from the immunolglobulin part of SDPP. They showed that SDPP gave a larger response in conventional nursery facilities than in a very high-health, environment controlled, off-site nursery.

The role of immunocompetence in enhanced pig performance is further suggested by the work of Stahly et al. (1994). They indicated that SDPP enhanced both rate and efficiency of growth in pigs with a high degree of antigen exposure, but not in pigs with a low degree of antigen exposure. The fact that SDPP is more effective than SDBP (Hansen et al., 1993: Smith et al., 1994) further suggests an involvement of the immune system of the animal and the immunoglobulin fraction of SDAP.

Other modes of action have been proposed and are discussed in the excellent review article by van Dijk et al. (2001). The above review also summarizes many growth experiments using spray dried plasma. Readers are also encouraged to peruse the review by Campbell (1998) that summarizes several feeding trials as well as processing, etc.

Potential Plasma Substitutes

Grinstead et al. (2000) presented results from five separate experiments comparing a concentrated whey protein product (WPP) containing 73% CP with SDAP. They concluded that WPP can be used in combination with or as a total replacement for SDAP in diets for weanling pigs without reducing performance. Close examination of their data show that the WPP response is much more variable than the response from SDAP. Gain of weanling pigs fed WPP did not equal those of pigs fed SDAP in two of the five experiments.

Virtually all of the experiments discussed in the SDAP section of this report show that neither dried skim milk nor dried whey (DW) are equal to SDAP for performance enhancements. Almost all of the research reported showing a SDAP response was in experiments where either DSM and/or DW were replaced.

Fish meal often used in weanling pig diets has been considered to be a high quality protein and certainly complements SDAP in those diets providing a highly digestible source of amino acids including the sulfur containing amino acids. Bergstrom et al. (1997) conducted two experiments comparing SDAP and select menhaden fish meal in diets for early weaned pigs (12-14 days of age). Fish meal was equivalent to SDAP in high health states pigs reared in SEW facilities, but SDAP was superior to fish meal when pigs were raised in a conventional all-in/all-out on-site nursery. The SDAP response was apparently greater in the on-site nursery compared to the SEW nursery again suggesting that immune function and health status are involved in the mode of action of SDAP. Nessmith et al. (1997) have reported indirect evidence to show that a fish meal-casein mixture was not as efficacious as SDAP in weanling pig diets. In their work, the substitutions were made on an isolysinic basis.

Some research has been conducted to compare egg products/by-products with SDAP in pig starter diets. Schmidt et al. (2003) reported that neither spray dried technical albumen (SDTA) or spray dried whole egg supported performance equal to SDAP in 17 d of age weaned pigs. Actually replacing 7.5% SDAP with 7.5% SDTA decreased performance. Zimmerman (1999) reported that substituting an inedible egg by-product (a mixture of whites and whole eggs) for SDAP in pig starter diets actually depressed gain and gain:feed ratio. James et al. (1999) found that a similar inedible egg product was a good source of amino acids in starter diets, but did not enhance performance as did SDAP.

To the author's knowledge, vegetable sources of protein have never been shown to be equal to SDAP. Chae et al. (1999) compared soybean meal, dried skim milk, isolated soy protein and wheat gluten with SDPP. The wheat gluten product (80% CP) did support performance equal to dried skim milk and was better than either soybean meal or isolated soy protein, but was inferior to SDPP.

Owusu-Asiedu et al. (2003) have shown that egg yolk antibody from hens immunized with *E. coli* K88 antigen was effective in pigs challenged with the K88 strain. SDPP also provided passive control of the *E. coli* infection.

Providing antibodies with this type of approach may be necessary if a true replacement for SDPP is to be found. It may be possible, utilizing some of today's molecular biological techniques, to engineer animals (milk, eggs) or microorganisms to produce the immunocompetence factors currently supplied by SDAP.

References Cited

Bergström, J.R., J.L. Nelssen, M.D. Tokach, R.D. Goodband, S.S. Dritz, K.O. Owen and W.B. Nessmith, Jr. 1997. Evaluation of spray-dried animal plasma and select menhaden fish meal in transition diets of pigs weaned at 12 to 14 days of age and reared in different production systems. J. Anim. Sci. 75:3004-3009.

Campbell, J.M. 1998. The use of plasma in swine feeds. *Discoveries, A Quarterly Tech. Update.* American Protein Corp., Ames. IA.

Chae, B.J., I.K. Han, J.H. Kim, C.J. Xang, J.D. Hancock, I.H. Kim and D.A. Anderson. 1999. Effects of dietary protein sources on ileal digestibility and growth performance for early-weaned pigs. Livestock Prod. Sci. 58:45-54.

Coffey, R.D. and G.L. Cromwell. 1995. The impact of environment and antimicrobial agents on the growth response of early weaned pigs to spray-dried porcine plasma. J. Anim. Sci. 73:2532-2539.

Ermer, P.M., P.S. Miller and A.J. Lewis. 1994. Diet preference and meal patterns of weanling pigs offered diets containing either spray-dried porcine plasma or dried skim milk. J. Anim. Sci. 72:1548-1554.

Gatnau, R. and D.R. Zimmerman. 1990. Spray dried porcine plasma (SDPP) as a source of protein for weanling pigs. J. Anim. Sci. 68(Suppl. 1):374.

Gatnau, R., C. Cain, M. Drew and D. Zimmerman. 1995. Mode of action of spray-dried porcine plasma in weanling pigs. J. Anim. Sci. 73(Suppl. 1):82.

Grinstead, G.S., R.D. Goodband, S.S. Dritz, M.D. Tokach, J.L. Nelssen, J.C. Woodworth and M. Molitor. 2000. Effects of a whey protein product and spray-dried animal plasma on growth performance of weanling pigs. J. Anim. Sci. 78:647-657.

Hansen, J.A., J.L. Nelssen, R.D. Goodband and T.L. Weeden. 1993. Evaluation of animal protein supplements in diets of early-weaned pigs. J. Anim. Sci. 71:1853-1862.

James, B.W., J.C. Sparks, M.H. Jurgens and D.R. Zimmerman. 1999. Comparison of inedible egg product and spray-dried plasma as sources of protein for weanling pigs. Swine Research Report ASL-R1658. Iowa State University, Ames, IA.

Nessmith, W.B., Jr., J.L. Nelssen, M.D. Tokach, R.D. Goodband, J.R. Bergström, S.S. Dritz and B.T. Richert. 1997. Evaluation of the interrelationships among lactose and protein sources in diets for segregated early-weaned pigs. J. Anim. Sci. 75:3214-3221.

Owen, K.O., J.L. Nelssen, R.D. Goodband, M.D. Tokach, K.G. Friesen, B.T. Richert, J.W. Smith and L.E. Russell. 1995. Effects of various fractions of spray-dried porcine plasma on performance of early weaned pigs. J. Anim. Sci. 73 (Suppl 1):81.

Owusu-Asiedu, A., C.M. Nyachoti, S.K. Baidoo, R. R. Marquardt and X. Yang. 2003. Response of early-weaned pigs to an enterotoxigenic *Escherichia coli* (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk antibody. J. Anim. Sci. 81:1781-1789.

Pierce, J.L., G.L. Cromwell, M.D. Lindemann and R.D. Coffey. 1995. Assessment of three fractions of spray-dried porcine plasma on performance of early-weaned pigs. J. Anim. Sci. 73(Suppl.):81.

Schmidt, L.S., C.M. Nyachoti and B.A. Slominski. 2003. Nutritional evaluation of egg by products in diets for early-weaned pigs. J. Anim. Sci. 81:2270-2278.

Smith, J.W., II, B.T. Richert, W.B. Nessmith, Jr., J.L. Nelssen, R.D. Goodband and M.D. Tokach. 1995. The effect of spray-dried plasma source on starter pig performance. J. Anim. Sci. 73(Suppl. 1):80.

Stahly, T.S., D.R. Cook, S.G. Swenson, N.H. Williams and D.R. Zimmerman. 1995. Growth responses of pigs to dietary plasma protein (PP) additions as influenced by pig antigen exposure to PP source. J. Anim. Sci. 73(Suppl. 1):81.

van Dijk, A.J., H. Everts, M.J.A. Nabuurs, R.J.C.F. Margry and A.C. Beynen. 2001. Growth performance of weanling pigs fed spray-dried animal plasma: A review. Livestock Prod. Sci. 68:263-274.

Weaver, E.M., L.E. Russell and M.D. Drew. 1995. The effect of spray-dried animal plasma fractions on performance of newly weaned pigs. J. Anim. Sci. 73(Suppl. 1):81.

Zimmerman, D.R. 1999. Effect of inedible egg product on growth performance of weanling pigs. Swine Research Report ASL-R 1659. Iowa State University, Ames, IA.

Feeding and Management Strategies for Successful Wean-to-Finish Pig Production

Bradley F. Wolter
The Maschhoffs, Inc.
Carlyle, IL 62231
618-594-2125
bradleyw@pigsrus.net

Summary

Rearing pigs in the same facility from weaning to market has gained popularity with pig producers across the globe in the last decade, and is commonly referred to as "wean-to-finish" production. Producers continue to adopt this system of management largely because it provides increased flexibility to animal flows with the potential to stabilize animal health status, as well as increase the ease of managing routine feeding and watering practices. A number of facility design and animal management factors may influence the growth performance achieved by pigs reared in a wean-to-finish system. Specifically, feeding and management strategies employed during the early growth period immediately post-weaning may have a significant impact on rate of growth and morbidity of pigs reared in a wean-to-finish system. The focus of this paper is to review recent research evaluating the impact of different strategies of rearing and management during early stages of development on overall growth performance of pigs from weaning to market in wean-to-finish production systems.

Introduction

Over the last decade, a single building designed to accommodate pigs from weaning to market in the same facility has been developed to replace more traditional multiple-stage housing systems. This "wean-to-finish" system has been introduced to pig managers largely because of the reduction in movement of the pig over its lifetime, and therefore a decrease in labor and risk of disease exposure during movement. Perhaps most important, research evaluating the rearing of pigs in either a traditional multiple-stage or single-stage (i.e., wean-to-finish) housing system found a similar level of growth performance among production systems (Brumm et al., 2002). Therefore, wean-to-finish production systems are increasingly being adopted by modern pig producers with the intention of rearing pigs with the potential for high protein deposition in technically engineered housing facilities using complex nutritional strategies. The typical wean-to-finish facility design is closely associated with a standard finishing house, but has specialized features to accommodate weanling piglets. Therefore, management strategies that may be unique to traditional production systems are largely found during the early period post-weaning. This paper will focus primarily on research evaluating the impact of different strategies of rearing and management during early stages of development on overall growth performance of pigs from weaning to market in a wean-to-finish production system. A more complete understanding of the relationship between the growth of pigs at specific stages of development on overall growth to market as influenced by feed resources, pig genetic potential, facility design and management, among key economic factors is necessary for the implementation of management strategies that maximize a given system's profitability.

Facility Features and Operation

Wean-to-finish facilities must accommodate growth and development of the pig across a wide range of body weight (BW) from weaning (e.g., 5 kg) to market (e.g., 130 kg). Key features of wean-to-finish facilities that provide successful pig growth performance include; feeders that maximize early feed intake and minimize later feed wastage, supplemental

heating and comfort zoning, ventilation systems that provide for optimal air exchange and minimize drafts, and equipment and facility layout that provides for rapid and thorough clean-up. The addition of these features to the traditional finishing facility not only allow for wean-to-finish pig rearing, but result in increased expense to the construction and operation of finishing facilities. One disadvantage of the wean-to-finish system is the considerable underutilization of floor space during the early growth period if pigs are penned in groups that are appropriate for finishing pigs. Therefore, a key to maximizing the output of the facility is determining an appropriate stocking rate (i.e., total number of animals/ unit of facility resource).

Over-Stocking During the Early Growth Period

The practice of placing more pigs into pens immediately at weaning (i.e., overstocking), with some of the pigs subsequently being moved to another finishing accommodation, is often used in order to increase output from a wean-to-finish facility. A summary of results on the impact of double stocking during the early period in wean-to-finish is presented in Table 1. The rate of mortality and morbidity did not statistically differ among stocking-rate treatments in the studies reviewed, however in practice, mortality rate during the early growth period appears to slightly increase (~0.5%) when doubling the initial stocking rate which may, in part, be explained by differences among the health status between pigs under research and commercial conditions. Among the reported studies, the environment experienced by the pigs differed in a number of important respects, including group size and floor and feeder space. In practice, each specific factor for a wean-to-finish facility such as ventilation rate, and feeder-and floor-space allocation and its relationship to pig growth performance should be considered to determine optimum duration of overstocking. In general, research results suggest the practice of overstocking for an initial eight to ten week period post-weaning reduces early growth, but has the potential to increase output from a wean-to-finish facility.

Access to Nutritional Resources

The amount of feeder and water drinker resources needed to support animal growth performance should be given consideration in designing wean-to-finish accommodation, and in determining its optimum management strategy. Competition among animals arises when a limitation, either in quantity, spatial distribution, or preference availability, of a nutritional resource (i.e., feed or water) exists. European researchers have shown an increase in the amount of feeder-related aggression and a reduction in growth rate when growing pigs are provided a limited feeder-space allocation (Spoolder et al., 1999). Similarly, Wolter et al. (2002b) determined that doubling the number of pigs per pen for eight weeks post-weaning in a wean-to-finish facility required additional feeder-trough space to maintain performance. In that study, the authors found that pigs provided 2 compared to 4 cm of feeder-trough space (Jumbo Wean-to-Finish Feeder, Farmweld, Teutopolis, IL) per animal had similar performance for the first 6 weeks post-weaning, however, pigs allocated to the reduced trough space had a 5% reduction in growth rate for the subsequent period between 6 and 8 weeks post-weaning. Results of a recent study conducted in our system using a tube-type combination wet/dry feeder (Aqua Tube Wean-to-Finish Feeder, Swine Service Specialist, Lyons, NE) are presented in Table 2. Results suggest providing over-stocked pigs supplemental feeder-trough space (5 vs. 3 cm/pig [4 vs. 2 tube spaces]) tends to increase growth rate during the four-week period immediately post-weaning. To that end, pigs that are over-stocked immediately post-weaning in wean-to-finish accommodation designed for achieving optimal performance during the finishing period may require additional feeder-trough space to maximize growth performance.

Grouping Pigs Upon Placement

The increase in size of operations and changes in design of facilities has created opportunities to put together large groups of pigs of uniform age and/or size. Consequently, the use of large group sizes of 100 or more pigs per group are of commercial interest to producers as one approach to lower housing cost and maximize facility use. Several studies have shown a negative impact of large group size (e.g., 20 vs. 100 pigs/pen) on growth rate of pigs during the nursery period (Wolter and Ellis, 2002). Similarly, pigs in a wean-to-finish facility kept in groups of 50 and 100 compared to 25 were found to have poorer growth performance during the initial eight weeks post-weaning, however, overall, for the period from weaning to slaughter, pigs had similar levels of performance for all three group sizes (Wolter et al., 2001). To that end, group size may, in part, explain

the reduction in growth rate experienced by pigs that are overstocked in the initial period after weaning in a wean-to-finish facility. It is also important to note, many caretakers feel that it is more difficult to identify unthrifty pigs as group size increases and this could lead to predisposing larger groups to higher levels of mortality and morbidity. Ultimately, the decision on the most appropriate group size to use in a particular wean-to-finish situation should be made on the basis of production costs and ease of management.

Managing the variation in pig body weights within a pen or house at market is of significant practical relevance to pig unit managers in their attempt to optimize facility utilization and maximize individual animal value. The marketing strategy desired should be considered at the time of weaning to account for sources of body weight variation (i.e., gender, weaning weight, or health status) when placing pigs into pens in order to optimize facility utilization during the finishing period as pigs will likely not be moved or sorted again prior to market. The strategy for marketing pigs from a wean-to-finish unit will be largely dependent on the individual production system, however, many producers remove portions of pigs from pens over an extended period of time during the finishing period to target a fixed market weight. In theory, placing pigs in pens to achieve a random body weight distribution within a pen at weaning will allow for removing an equal number of pigs from each pen during the marketing period and, therefore, may optimize overall facility utilization. In support of this strategy, Wolter et al. (2002b) found that sorting pigs into pens so as to minimize variation in body weight within each pen has little impact on either subsequent growth rate or variation in body weight at market. However, the opportunity to sort heavier and lighter pigs by house when moving portions of overstocked pigs to different finishing accommodation after the nursery period can decrease the total time required to empty each house when targeting a fixed market weight. Nevertheless, under conventional stocking rates the practice of sorting pigs into pens in an attempt to minimize variation within each pen does not appear to impact growth rate and, therefore, holds little potential to increase total throughput of a production system.

Feeding Management

At weaning, piglets must adapt to considerable changes in their environmental, immunological, and nutritional status associated with the rapid shift away from resources provided by the sow to those provided by human caretakers. Research has demonstrated the abrupt changes often seen by the piglet at weaning can have a significant impact on their growth performance dependant on caretaker management of the weaning process (McCraken et al., 1995; Wolter and Ellis, 2001). Piglet management strategies aimed at increasing feed intake can result in improved growth performance of newly weaned pigs and may reduce animal losses (Corrigan, 2002; Haag et. al., 2004). The provision of a clean, sanitized, warm, dry, and draft free environment should be a component considered by any management strategy aimed at enhancing feed intake and promoting high health of the pig at weaning (Scheepens, 1991). In addition, facility design and feeding equipment must provide a simple means to implement feeding strategies that enhance piglet feed intake in an effort to assist the animal in maintaining growth rate during the transition at weaning.

Feed Form and Placement

Prior to weaning, piglets, as grouped with littermates, consume primarily a liquid-milk diet in small, frequent feeding bouts. Therefore, it may be advantageous for pig unit managers to attempt to facilitate these behaviors by frequently feeding small quantities of the weanling diet four to six times per day to stimulate feed intake during the weaning process (Corrigan, 2002). Placement of supplemental feed, in addition to that provided in an ad-libitum feeder, onto a floor mat or into a feeder trough has been demonstrated to increase piglet growth rate during the period immediately post-weaning in a wean-to-finish facility (Corrigan, 2002). However, studies evaluating the placement of feed on floor mats to encourage feed intake during the period immediately post-weaning have produced inconsistent results on pig performance (Mavromichalis et al., 2000; Augspurger et al., 2002). To that end, inconsistencies among the studies in effects of floor feeding may have been associated with differences in the form of diet fed, age of pigs at weaning, and body weight range of pigs evaluated in each study (Corrigan, 2002). In commercial practice, floor mat feeding appears to reduce the morbidity as defined by pigs appearing ill, diseased, or unthrifty,

characterized by loss of body weight (Corrigan, 2002). In support, a recent study conducted in our system found supplemental feeding strategies, either floor feeding or use of an automated gruel feeder where found to reduce the number of antibiotic treatments given to pigs by one-half compared to only the provision of an ad-libitum feeder during the initial period post-weaning (B. Peterson, Personal Communication). In general, a floor feeding protocol for weanling piglets should involve a solid floor area large enough to allow all pigs within a pen access to feed, and consider a volume of feed at each feeding that allows all pigs access but minimizes feed wastage. While the impact of floor feeding the diet in either a gruel or dry form on pig growth performance appears inconsistent, in practice, the routine observations of piglets as a result of multiple-time per day floor feeding can provide for more timely response to unexpected alterations in the wean-to-finish facility.

Diet Complexity

Research has demonstrated that feeding corn-soybean meal-based diets that minimize the inclusion of milk, processed cereals, animal protein-based ingredients (i.e., simple compared to complex diets) can restrict growth rate during the early growth period (Whang et al., 2000). The restricted growth rate observed in pigs fed simple diets results from both lower feed intake and lower feed efficiency (Wolter et al., 2002c). The increase in feed intake associated with increased diet complexity is most pronounced in the immediate post-weaning period (Dritz et al., 1996). Of practical relevance, results from research carried out in our production system demonstrate the rate of piglet morbidity can increase with decreases in diet complexity during the early growth period (Table 3). Consequently, in practice, feeding pigs simple diets immediately post-weaning may result in an increased use of therapeutic antibiotics as pigs exhibit signs of decreased feed intake and lethargy (Haag et al., 2004). Therefore, the selection of ingredients for use in piglet diets is important to maximizing the early growth potential and reducing the threat of health challenges or digestive disorders after weaning. However, increases in diet complexity of the dietary program frequently increase costs. Because the ingredients used to manufacture complex diets are relatively expensive, their effect on piglet performance both in the post-weaning and subsequent growth phases should be considered to optimize profitability.

A phase feeding strategy should be employed in order to gradually transition the pig's digestive system from a diet that is high fat, high lactose, and containing simple-animal proteins near weaning to a diet containing low fat, low lactose, and complex-plant protein. The primary objectives of the initial diet are to match dietary ingredients with the digestive capability of the pigs while enhancing intake of a dry form of diet. Simple diets can be used relatively quickly after the pig has begun eating a substantive volume of feed, and this strategy will allow for rapidly lowering diet cost while maintaining optimal growth performance.

Variation in Growth Rate During Early Stages and Overall Growth Performance

Throughout this discussion it is demonstrated that animal management factors can have a major impact on the growth and development of pigs in the early stages post-weaning. However, the effect of varied early growth and development on subsequent growth performance can be influenced by the stage of growth, physiological state, and genotype of the animal at the start of the period when growth is varied and the severity by which growth change, duration of altered growth, and nutrient composition and level of food intake during and after the period of varied growth (Wolter, 2002). To that end, Wolter (2002) in a series of studies conducted within wean-to-finish systems found that pigs that experience decreased early growth rate had increased growth and feed efficiency when provided adequate nutritional and special resources in the subsequent grow-finish period. Therefore, producers may be able to exploit the potential to reduce production costs by using strategies that manipulate the growth curve of the animal within a range of nutritional and environmental approaches. The effect of varied growth rate due to management of pigs in commercial practice on overall growth performance from weaning to market in conjunction with carcass composition should be used in place of independent growth periods after weaning to defining optimum management strategies for wean-to-finish facilities.

References

Augspurger, N. R., T. M. Parr, and D. H. Baker. 2002. The effect of floor feeding on post-weaning piglet weight gain. J. Anim. Sci. 80: (Suppl. 2): 135 (Abstr.).

Brumm, M.C., A. K. Baysinge, R. W. Wills, and R. C. Thaler. 2002. Effect of wean-to-finish management on pig performance. J. Anim. Sci. 80:309-315.

Corrigan, B. P. 2002. The effects of feeding management on growth performance and survivability of newly weaned pigs. M.S. Thesis. University of Illinois. USA.

DeDecker, J. M. 2002. Effects of space allowance in a wean-to-finish system and pig removal strategies at market on the growth performance and variation in performance of pigs. M. S. Thesis. University of Illinois. USA.

Dritz, S. S., K. Q. Owen, J. L. Nelssen, R. D. Goodband, and M. D. Tokach. 1996. Influence of weaning age and nursery diet complexity on growth performance and carcass characteristics and composition of high-health status pigs from weaning to 109 kilograms. J. Anim. Sci. 74:2975-2984.

Haag, A., B. Payne, J. F. Lowe, B. F. Wolter, and D. M. Webel. 2004. Impact of diet complexity on growth performance and morbidity of pigs after weaning. Proc. Am. Assoc. Swine Vet. Annual Meeting, 2004, pp 53-57.

Mavromichalis, I., J. E. Stoerger, and D. H. Baker. 2000. Floor feeding enhances early appetite of weaned pigs. J. Anim. Sci. 78 (Suppl. 1):31 (Abstr.).

McCracken, B. A., H. R. Gaskins, P. R. J. Ruwe-Kaiser, K. C. Klasing, and D. E. Jewell. 1995. Diet-dependent and Diet-independent metabolic responses underlie growth stasis of pigs at weaning. J. Nutr. 125:2838-2845.

Scheepens, C. J. M. 1991. Effects of draught as climatic stressor on the health status of weaned pigs. Ph. D. Thesis. University of Utrecht. The Netherlands.

Spoolder, H. A. M., S. A. Edwards, and S. Corning. 1999. Effects of group size and feeder space allowance on welfare in finishing pigs. Anim. Sci. 69:481-489.

Whang, K. Y., F. K. McKeith, S. W. Kim, and R. A. Easter. 2000. Effect of starter feeding program on growth performance and gains of body components from weaning to market weight in swine. J. Anim. Sci. 78:2885-2895.

Wolter, B. F. 2002. Effects of variation in early growth rate of pigs on subsequent growth performance. Ph. D. Thesis. University of Illinois. USA.

Wolter, B. F. and M. Ellis. 2001. The effects of weaning weight and rate of growth immediately after weaning on subsequent pig growth performance and carcass characteristics. Can. J. Anim. Sci. 81:363-369.

Wolter, B. F., and M. Ellis. 2002. Impact of large group sizes on growth performance in pigs in the USA. Pig News and Info. 23:17N-20N.

Wolter, B. F., M. Ellis, J. M. DeDecker, S. E. Curtis, G. R. Hollis, R. D. Shanks, E. N. Parr, and D. M. Webel. 2002a. Effects of double stocking and weighing frequency on pig performance in wean-to-finish production systems. J. Anim. Sci.. 80:1442-1450.

Wolter, B. F., M. Ellis, S. E. Curtis, N. R. Augspurger, D. N. Hamilton, E. N. Parr, and D. M. Webel. 2001. Effect of group size on pig performance in a wean-to-finish production system. J. Anim. Sci. 79:1067-1073.

Wolter, B. F., M. Ellis, S. E. Curtis, E. N. Parr, and D. M. Webel. 2002b. Effects of feeder-trough space and variation in body weight within a pen of pigs on performance in a wean-to-finish production system. J. Anim. Sci. 80:2241-2246.

Wolter, B. F., M. Ellis, S. E. Curtis, E. N. Parr, and D. M. Webel. 2002c. Impact of early postweaning growth rate as affected by diet complexity and space allocation on subsequent growth performance of pigs in a wean-to-finish production system. J. Anim. Sci. 81:353-359.

Table 1. Summary of data on the effect of stocking rate on pig growth performance during the initial period after weaning in wean-to-finish facilities.

Stocking rate	Body weight range, kg	Group size, no. pigs	Feeder-trough space, cm/pig	Floor space, m^2/pig	Response to double stocking (percentage unit difference)		
					ADG	ADFI	G:F
Wolter, Unpublished data							
Single	4.5 to 32.3	32	5.8	0.640			
Double	4.5 to 30.3	64	2.9	0.320	- 7.3	-	-
DeDecker, 2002							
Single	4.8 to 45.0	27	5.6	0.640			
Double	4.8 to 41.7	54	2.8	0.320	- 7.9	-	-
Wolter et al., 2002a							
Single	5.8 to 42.6	52	4.0	0.650			
Double	5.9 to 39.7	104	2.0	0.325	- 7.7	- 7.0	+ 1.8
Brumm et al., 2002							
Single	5.1 to 28.7	15	4.8	0.690			
Double	5.1 to 26.9	30	2.4	0.350	- 7.1	- 7.0	0.0

Table 2. Effects of initial stocking rate and feeder space on pig growth performance in a wean-to-finish facility.

Initial stocking rate[a]	Single		Double		
Feeder-trough space[b]	Control	Supplemental	Control	Supplemental	SEM
Pigs, no.	160	160	320	320	
Weight, kg					
Weaning	4.5	4.5	4.5	4.5	0.02
Week 4[c]	14.2[e]	14.2[e]	13.7[f]	14.1[e]	0.14
Week 6[c]	21.2[e]	21.1[e]	19.8[f]	20.7[e]	0.25
Week 8[c]	32.3[e]	32.4[e]	30.3[g]	31.4[f]	0.32
Average daily gain, g					
Weaning through week 4[c]	338[e]	335[e]	318[f]	332[e]	5.0
Week 5 through week 6[d]	535	531	472	503	14.1
Week 7 through week 8[d]	726	735	685	708	18.1
Weaning through week 8[c]	490[e]	490[e]	454[g]	472[f]	5.9

[a] Initial stocking rate = Single (32 pigs/pen) and Double (64 pigs/pen).
[b] Feeder-trough space = Control (183 cm; 2 tubes available) and Supplemental (305 cm; 4 tubes available).
[c] Initial stocking rate and feeder-trough space interaction response ($P < 0.10$).
[d] Initial stocking rate response ($P < 0.10$).
[e, f, g] Within a row means without a common superscript letter differ ($P < 0.10$).

Table 3. Effect of diet complexity on pig growth performance, morbidity rate, and incidence of therapeutic treatment during an initial period post-weaning in a commercial wean-to-finish system.[a]

	Dietary treatment[b]			
	Complex	Simple	SEM	P-Value
Feeders, no.[c]	8	4	–	–
Body weight, kg				
Weaning	4.4	4.4	0.02	0.67
Day 5 post-weaning	5.0	4.8	0.06	0.13
Day 12 post-weaning	6.7	6.3	0.07	0.01
Day 21 post-weaning	9.3	8.8	0.23	0.30
Growth performance				
Weaning through day 5				
ADG, g	113	80	10.6	0.11
ADFI, g	162	140	11.1	0.36
Gain:feed, g:g	0.73	0.58	0.056	0.16
Day 5 through day 12				
ADG, g	259	232	10.1	0.17
ADFI, g	323	259	17.2	0.06
Gain:feed, g:g	0.81	0.82	0.025	0.88
Weaning through day 12				
ADG, g	198	155	7.2	0.01
ADFI, g	253	209	12.6	0.07
Gain:feed, g:g	0.79	0.75	0.014	0.15
Day 12 through day 21				
ADG, g	282	281	19.0	0.98
ADFI, g	460	413	12.3	0.06
Gain:feed, g:g	0.61	0.68	0.037	0.37
Weaning through day 21				
ADG, g	232	208	12.2	0.29
ADFI, g	342	295	11.4	0.04
Gain:feed, g:g	0.69	0.71	0.020	0.70
Removal rate, %[d]				
Weaning through day 21	0.9	3.1	0.09	0.03
Treatment, %				
Weaning through day 21	12.3	18.8	2.65	0.02

[a] Adapted from Haag et al., 2004.
[b] Dietary treatment = Complex (Milk product, raw and cooked cereal, and animal-protein based ingredients with minimal soybean meal) and Simple (Corn-soybean meal base with minimal levels of milk product and animal-protein based ingredients).
[c] Two pens of 28 pigs shared access to an in fence-line feeder.
[d] Pigs removed were characterized by the appearance of serious illness, disease, or unthrifty, and exhibiting a loss of body weight.

The Role of the Adipocyte in Energy Regulation

Michael E. Spurlock, Kolapo M. Ajuwon, and Sheila K. Jacobi
Department of Animal Sciences
Purdue University
West Lafayette, IN 47907
765-494-4820
spurloc0@purdue.edu

Summary

Since the pioneering parabiosis studies of 40 years ago provided the early indications that a circulating factor, produced in proportion to body fat mass, regulated feed intake and body fat reserves, the adipocyte has emerged as a pivotal regulator of energy balance. This cell is now a recognized endocrine cell, and two adipocyte-derived hormones, leptin and adiponectin, have been identified as key determinants of feed intake and energy expenditure. Leptin is produced by the adipocyte, but acts centrally and peripherally to regulate appetite and energy expenditure and coordinate whole body energy metabolism. Leptin acts as an energy balance and nutrient sensor, and its expression and function are regulated by endocrine and dietary factors. Leptin also regulates lipid metabolism, including lipogenesis, lipolysis, and fatty acid oxidation in multiple peripheral tissues.

Adiponectin is a relatively new "adipocytokine" that circulates in the blood as the mature protein, and as an apparent carboxyl-terminal cleavage peptide. Both adiponectin and the cleavage peptide regulate energy metabolism in skeletal muscle and liver, albeit with different potencies. Specifically, adiponectin causes weight loss, up regulates fatty acid transporters, stimulates fatty acid oxidation in skeletal muscle, and enhances the suppression of glucose production by insulin in the liver. However, a very intriguing and distinct feature of adiponectin is its direct connection to the immune system. This hormone acts directly on macrophages and adipocytes to suppress the production of certain cytokines, which are also potent regulators of energy metabolism in peripheral tissues. Thus, through adiponectin, the adipocyte acts as a bridge between energy balance and immune system, and the implications of this linkage extend not only to the regulation of energy metabolism under homeostatic circumstances, but also during periods of acute or chronic inflammation in which energy partitioning and must be effectively controlled. In light of the fact that chronic stress and immune stimulation causes an appreciable suppression of growth and efficiency in pigs reared commercially, this particular role of the adipocyte is receiving much attention.

Collectively, the adipocyte derived hormones are offering tremendous new insights into the endocrine regulation of energy balance, and therein, new targets for nutritional and genetic intervention strategies are emerging. Ultimately, these new technologies and strategies will enable pork producer to capture more value from the energy they feed, and the greater profitability will ensure industry viability.

Introduction

Improving growth rate, efficiency of gain, and product quality are primary research objectives for food animal scientists. To this end, it is quite exciting that the adipocyte is emerging rapidly as a significant *regulatory cell* that produces hormones and cytokines which influence energy metabolism locally and in peripheral tissues (Sethi and Hotamisligil, 1999). The complexity with which energy balance is regulated is now readily apparent, and is underscored by the recent discoveries pertaining to the integration of immune function with adipocyte biology and the regulation of energy metabolism. The *Toll-like receptors* (TLR) are pathogen recognition receptors

that ultimately stimulate the transcription of genes involved in the coordination of the inflammatory process. For example, TLR-2 recognizes protozoan substrates, TLR-4 recognizes bacterial motifs, and TLR-9 recognizes bacterial DNA. *The adipocyte expresses the bona fide membrane receptors for gram-negative and gram-positive organisms (TLR-4 and TLR-2, respectively), and responds to direct stimulation by producing proteins that augment or attenuate specific immunological processes* and energy metabolism (Lin et al., 2000; Ajuwon et al., 2004). Apart from direct participation in the acute phase response to infection, the adipocyte produces a number of proteins that regulate metabolism and immune response pathways. Leptin and adiponectin are two such proteins. These hormones have overlapping and distinct biological functions that encompass energy balance and immune function. In this paper, we will highlight the new findings regarding the adipocyte and the regulation of energy balance, and make particular applications to the efficiency of growth in commercial pork production endeavors.

Leptin

Leptin as a Nutrient Sensor. Leptin is produced largely in the adipose tissue (Zhang et al., 1994; Bidwell et al., 1997) and secreted into the blood. Apart from the central actions of leptin, *there are compelling indications of direct effects on peripheral tissues.* Leptin receptors have been identified in adipose tissue (Lollman et al., 1997; Lin et al., 2000), and regulation of this receptor may augment or attenuate the local actions of leptin. Intense study is underway to determine the mechanism(s) by which leptin "senses" energy balance. Linked to energy sensing is the notion that leptin serves as a nutrient sensor, specifically sensing nutrient flux in adipocytes and other cells. One way this could be accomplished is via changes in circulating insulin concentrations. Insulin levels in blood change rapidly in response to feeding and insulin has been shown to regulate leptin gene expression in many species (see reviews, Houseknecht and Portocarrero, 1998; Ahima & Flier 2000).

The regulation of leptin expression by changes in nutrient flux appears to involve more than changes in insulin concentrations, however. Work from several laboratories has implicated the hexosamine biosynthetic pathway in leptin's role as cellular nutrient sensor. Marshall et al. (1991) proposed that cellular hexosamine biosynthesis is an important mechanism by which cellular glucose flux in cells is sensed. UDP-N-acetylglucosamine, the end product of hexosamine biosynthesis, is used in O-linked glycosylation reactions; important regulatory steps in gene transcription. Specifically, the activity of some transcription factors is modified by O-linked glycosylation. Leptin expression in adipose tissue of humans (Considine et al. 2000) and rodents (Wang et al. 1998; Wang et al., 1999b; McClain et al., 2000; Obici et al. 2002b) is regulated by the hexosamine biosynthetic pathway, consistent with the observation that leptin expression in adipocytes *in vitro* is more sensitive to glucose concentrations than insulin (Mueller et al. 1998). It seems that the mechanisms by which hexosamine biosynthesis regulate leptin production involve the regulation of activity of transcription factors. In experiments using 3T3-L1 adipocytes transfected with a leptin gene promoter-luciferase reporter construct, Zhang et al. (2002) found that leptin production in 3T3-L1 adipocytes is stimulated by the metabolism of glucose to hexosamines via transcriptional mechanisms in the proximal promoter of the leptin gene.

Leptin: Energy Balance Sensor. The ability of exogenous leptin to regulate appetite and energy expenditure in rodents with subsequent loss of adipose tissue but not lean body mass has led to the notion that leptin is an anti-obesity hormone (see reviews: Friedman and Halaas, 1998; Houseknecht and Portocarrero 1998). Obesity, despite high circulating leptin concentrations in rodents and humans, has led to the notion of leptin resistance (discussed in detail, below). It has been proposed that obesity in the face of leptin resistance reflects the evolutionary advantage of being able to store excess energy as lipid during times of nutritional plenty (Flier 1998). This has led to the notion that leptin's primary physiological role is in sensing negative energy balance and coordinating physiological signals leading to whole-body adaptation to fasting (Flier, 1998; Ahima and Flier 2000). Studies from several groups (Ahima et al. 1996; Legradi et al 1997; Yu et al. 1997; Finn et al. 1998; Lord et al. 1998; Nagatani et al. 1998) have shown that indeed, prevention of the fasting-induced fall in leptin concentrations by the administration of exogenous leptin treatment during fasting is able to at least partially overcome the metabolic, endocrine, reproductive and immune system adaptations to starvation.

Nutritional Regulation of Leptin. As leptin has been implicated as a major sensor of energy and

nutrient balance it is not surprising that the leptin axis is regulated by nutritional status. The vast majority of published manuscripts examining the impact of nutrition on leptin expression and activity have focused on caloric intake, namely comparison of *ad libitum* feeding of highly palatable, and often high fat diets vs. low-fat diets. Additionally, a large body of literature has compared effects of *ad libitum* feeding to food deprivation (acute or chronic fasting) on leptin expression. Data in species from rodents to livestock to humans have shown that chronic overconsumption of calories leads to increased leptin gene expression coincident with increasing adiposity (see reviews: Houseknecht and Portocarrero, 1998; Ahima & Flier 2000; Barb et al. 2001). Likewise, many laboratories have shown that fasting results in significant down-regulation of leptin expression in many species. Indeed, food deprivation has a more profound and immediate impact on leptin expression compared to chronic overfeeding. Although relatively few in number, studies examining the role of specific nutrients on leptin gene expression and activity are beginning to appear in the published literature. Those studies will be the focus of this review.

Leptin Regulates Lipogenesis and Lipolysis in Preadipocytes and Adipocytes. A schematic model of leptin action in the adipocyte is shown in Figure 1. In rodent models, leptin acts directly to suppress the inhibition of ß-adrenoceptor-mediated lipolysis by insulin, and simultaneously reduces insulin-induced glucose transport and lipogenesis (Müller et al., 1997), and similar effects have been reported in porcine adipocytes derived from stromal-vascular cultures (Ramsay, 2001). However, leptin also acts directly to stimulate lipolysis in adipocytes in the absence of insulin (Frühbeck et al., 1997, 1998; Ajuwon et al., 2003). Mechanistically, several things must be considered. Hormone sensitive lipase (HSL) is activated by protein kinase A as a result of the production of cAMP by adenylate cyclase, and is the major mediator of lipolysis within the adipocyte (see Ramsay, 1996). Although the effect of leptin on the expression of HSL in adipose tissue has been addressed in two rodent studies, one in which expression was increased (Sarmiento et al., 1997), and one in which it was not (Scarpace et al., 1998), the regulation of the adenylate cyclase-cAMP system has not been studied. Leptin expression itself is down regulated by cAMP (Slieker et al., 1996), and in the study of Wang et al. (1999a), leptin stimulated lipolysis and also decreased leptin expression. If norepinephrine, a potent inducer of cAMP production, had reduced leptin expression, one might conclude that leptin acts solely through the production of cAMP to activate lipolysis. However, this was not the case. Recent work published by Frühbeck et al. (2001) indicates that leptin antagonizes the tonic inhibition of lipolysis by adenosine. Leptin is a member of the Class-I family of helical cytokines (Madej et al., 1995), as is growth hormone. It is thus interesting that growth hormone, with similarities to leptin in receptor signaling (Tartaglia et al., 1995), alters the functionality of the G_i (i.e., diminished ADP-ribosylation by pertussis toxin) in adipose tissue (Houseknecht and Bauman, 1997). In this study, the diminished functionality of G_i was associated with an enhancement of lipolysis by growth hormone. Although similar results were not obtained in ovine adipocytes, growth hormone nonetheless antagonized the anti-lipolytic action of an adenosine analog, an effect likely stemming from a stifled interaction between the G_i α subunit and adenylate cyclase (Doris et al., 1998).

Leptin Causes Fatty Acid Oxidation, Adipose Ablation, and Apoptosis in Adipose Tissue. Early work with models in which hyperleptinemia was induced (Chen et al., 1996; Shimabukuro et al., 1997) indicated that specific adipose depots were virtually obliterated. Furthermore, the recovery of body fat in hyperleptinemic rats was much slower than in diet-matched controls (Higa et al., 2000). The implications of these results were that there was a sustained impact of hyperleptinemia on the ability of adipocytes to accumulate and store lipid. A portion of this response is possibly related to an induction of apoptosis and loss of adipocytes caused by elevated leptin concentrations within the brain (Qian et al., 1998b), if the higher circulating concentrations also achieve higher concentrations within the CNS. However, the majority of the response is likely due to an induction of fatty acid release, and concomitant up regulation of genes that regulate fatty acid oxidation (i.e., acyl CoA oxidase and carnitine palmitoyl transferase) that is driven by increased PPARα expression (Wang et al., 1999). In fact, an upregulation of PPARα has recently been shown to be a prerequisite for depletion of body fat by leptin. Using wild type and PPARα$^{-/-}$ mice, Lee et al. (2002) determined that the depletion of body fat in the null mice during hyperleptinemia is markedly lower than in wild-type mice. This effect was associated in part with the failure of hyperleptinemia to achieve an upregulation of carnitine palmitoyl transferase in adipose tissue due to the absence of PPARα.

The physiologic changes associated with hyperleptinemia shift the metabolic goal of adipocytes from lipid storage to lipid disposal. Also of considerable significance is that leptin ultimately results in a dedifferentiation of adipocytes (Zhou et al., 1999), as indicated by the loss of adipocyte marker (aP2) and concomitant appearance of the preadipocyte marker, Pref-1. Collectively, these findings indicate that leptin may be used to reduce adipose mass, but it is important to note that these extreme results are achieved only with pharmacological circulating concentrations of leptin (Higa et al., 2000), and are not indefinite.

Although the most pronounced effects of leptin on body fat are achieved with pharmacological blood levels, significant shifts in metabolism have been noted under less extreme conditions. Specifically, Chen and Heiman (2000; 2001) have shown in rodent models that daily peripheral injections of leptin caused a sustained stimulation of lipid utilization that was reflected in reduced plasma NEFA and triglyceride concentrations, and in lower respiratory quotients relative to controls. Furthermore, Reidy and Weber (2002) determined in rabbits that a single i.v. leptin bolus stimulated lipolysis (increased serum NEFA concentrations) and enhanced triglyceride-fatty acid cycling as a means of energy expenditure.

The mechanism for this critical role of leptin is such that there is a coordinated shift in metabolism from storage to oxidation of fatty acids. Generally, this transition from storage to oxidation involves an enhancement of systems that transport and oxidize fatty acids, and a repression of lipogenic systems similar to what is seen in adipocytes. However, the principal mechanism differs by tissue, and seems to reflect whether preexisting oxidative machinery within a given cell type is capable of handling the increased oxidation of lipid brought about by leptin (Lee et al., 2001).

Skeletal muscle constitutes the major proportion of whole body mass, and is an important determinant of metabolic rate and energy metabolism (Zurlo et al., 1990). There is an intriguing relationship between leptin and lipid metabolism emerging in skeletal muscle that is, overall, quite consistent with the concept that this hormone regulates lipid storage in non-adipocytes and also stimulates skeletal muscle thermogenesis. Early work indicated that leptin acts on skeletal muscle to attenuate the pro-lipogenic actions of insulin (Muoio et al., 1997), and to stimulate fatty acid oxidation while diminishing triglyceride accumulation (Muoio et al., 1997, 1999; Steinberg et al., 2002a). Subsequent work has established that with chronic administration of leptin (two weeks), there is a sustained enhancement of fatty acid oxidation and triglyceride hydrolysis in rat muscle that is apparent in both the resting and contracting states (Steinberg et al., 2002b). Additional work from this group (Steinberg et al., 2002c) now indicates that chronic leptin administration also diminishes fatty acid transport capacity by reducing the abundance of both the fatty acid translocase and fatty acid binding protein in plasma membrane fractions of red and white muscles. The activity of the 5'-AMP-activated protein kinase (AMPK) seems to be a critical component by which leptin stimulates fatty acid oxidation in skeletal muscle. Recent work (Minokoshi et al., 2002) has established quite clearly that leptin activates this kinase in skeletal muscle, which monitors intracellular fuel status, and promotes fatty acid oxidation by inhibiting lipogenic activity at the level of acetyl-CoA-carboxylase. Although the long-term regulation of this enzyme by leptin may be mediated through a central mechanism, the acute regulation is a direct effect on the skeletal muscle. It is also of interest that this particular mechanism for leptin may be specific to skeletal muscle. Others (Atkinson et al., 2002) determined that whereas leptin also stimulates fatty acid oxidation in cardiac muscle, the effect is achieved independent of activation of the AMPK.

Adiponectin

Discovery of adiponectin and its biological implications. Adiponectin, also known as Acrp30, AdipoQ, Apm1, and GBP-28, is an adipocyte-derived hormone that was discovered by several groups across multiple species almost simultaneously (Maeda et al., 1996; Scherer et al., 1996; Hu et al., 1996; Nakano et al., 1996). Adipocytes, and preadipocytes to a lesser extent, are typically the only source of adiponectin (Scherer et al., 1995). The expression of this protein is up-regulated by feed deprivation (Berg et. al. 2001) and cold exposure (Yoda et al., 2001), and is down-regulated in conditions of obesity (Arita et al., 1999). Adiponectin circulates in the blood largely as the mature protein. However, Fruebis et al. (2001) identified the carboxyl terminal fragment, which is most likely a proteolytic cleavage peptide derived from the mature protein, as a normal component of human plasma. We have obtained evidence of a similar cleavage peptide in the pig (un-

published data). Although is not yet known whether the production of the cleavage peptide is regulated by specific metabolic or immunologic circumstances, both the intact protein and cleavage peptide regulate lipid and glucose metabolism in peripheral tissues.

Metabolic activity of adiponectin. Thus far, there is little published information regarding the regulation of energy metabolism by adiponectin in swine or other meat animals. We have determined that adiponectin suppresses lipogenesis in primary pig adipocytes, and that this occurs with physiological concentrations (Jacobi et al., 2004). Other investigations of the metabolic roles of adiponectin have been focused on its relationship to obesity and insulin sensitivity in liver and skeletal muscle. Arita et al. (1999) determined that circulating adiponectin concentrations are lower in obese individuals, and in association with non insulin-dependent diabetes, than in individuals with normal body composition and insulin sensitivity. This finding has been of particular interest to researchers because it is in stark contrast with the regulation of leptin, which is markedly up-regulated in conditions of obesity. Recently, Fruebis et al. (2001) provided compelling evidence that adiponectin regulates lipid metabolism and body composition. Using recombinant adiponectin and a carboxyl terminal peptide produced by trypsin cleavage of the recombinant protein (gAd), these researchers identified three key metabolic implications for adiponectin. First of all, the post-prandial surges in plasma triglyceride, free fatty acid, and glucose concentrations were blunted relative to controls when gAd was administered to mice consuming a high-fat, sucrose diet, or infused (i.v.) with a fat emulsion. Secondly, it was determined that gAd stimulates free fatty acid oxidation in skeletal muscle preparations, and in cultured C2C12 myotubes. Finally, the authors tested the effect of gAd on the growth of immature mice, and on the weight change of mature mice, all of which were fed the high-fat, sucrose diet. In short, after an initial weight loss, weight gain was suppressed in the young growing mice, and appreciable weight loss was induced and sustained in the mature mice, in response to exogenous gAd. Although not determined directly, the implication is that adiponectin (i.e., gAd) acts to suppress fat accretion, and promote utilization, rather than storage, of body fat in growing and mature animals. It is perhaps quite significant that our western blot analyses indicate that serum adiponectin concentrations are substantially higher in a high-lean line of pigs than in a much fatter line (Jacobi et al., 2004).

Two recent publications provide considerable insight as to how adiponectin may be acting upon the adipocyte to regulate lipogenesis, as our data clearly indicate it does. Independent laboratories (Yamauchi et al., 2002; Tomas et al., 2002) have shown convincing evidence that the linkage of adiponectin to the control of fatty acid oxidation and triglyceride storage in skeletal muscle is through the 5'-AMP-activated protein kinase (AMPK). Briefly, the adiponectin cleavage peptide (i.e., gAd), and adiponectin itself to a lesser extent, activates the AMPK. With such activation, acetyl Co-A carboxylase is phosphorylated and thereby deactivated. Consequently, cytosolic malonyl Co-A is depleted, its repression of carnitine palmitoyltransferase is relieved, and fatty acid transport and oxidation are thus increased. Importantly, both isoforms of acetyl Co-A carboxylase (i.e., ACC_α and ACC_β) are targets of the AMPK (Yamauchi et al., 2002). It seems likely that adiponectin or gAd activates the AMPK in the pig adipocyte, and thereby attenuates lipogenesis and stimulates fatty acid oxidation. Additional support for our hypothesis relates to the regulation of NFkB signaling by adiponectin in adipocytes. We have shown that adiponectin attenuates the translocation of NFkB to the nucleus of adipocytes stimulated with lipopolysaccharide (Ajuwon and Spurlock, 2004). This is an important observation because a chemical activator of AMPK (AICAR), or expression of a constitutively active AMPK (Ruderman et al., 2003), disrupts NFkB-mediated gene expression in some cell types. Accordingly, if adiponectin activates the AMPK, a reduction in NFkB-mediated gene expression, presumably due to disrupted translocation to the nucleus, would be expected and the expression of PPARα increased. This is particularly important as PPARα is central to the regulation of fatty acid metabolism in adipocytes and skeletal muscle.

Adiponectin suppresses proinflammatory cytokine expression in activated immunocytes and adipocytes. Yokota et al. (2000) first reported that adiponectin suppressed proinflammatory cytokine production in activated human macrophages. We have recently reported similar results for both TNFα and IL6 in activated pig macrophages (Wulster-Radcliffe et al., 2004) and human THP-1 monocytes (Wulster-Radcliffe et al., 2004b). These findings undoubtedly reflect the integration of regulatory pathways for acute inflammation with those of energy metabolism. The latter point is further reinforced in that our laboratory has also found that adiponectin suppresses IL-6

expression in pig adipocytes stimulated with LPS (Ajuwon and Spurlock, 2004). Additional support for the inhibitory action of adiponectin on proinflammatory cytokine production stems from an adiponectin knockout model. Using this knockout model, Maeda et al. (2002) showed that adipose expression of TNFα was higher in the absence of adiponectin, and that the increased expression coincided with increased circulating concentrations of this cytokine. Even more striking was the finding that administration of adiponectin to the knockout mice abrogated the increase in adipose expression of TNFα, and lowered the circulating concentration to wild type levels. These findings, coupled with previous work showing that TNFα reduces adiponectin expression (Ruan and Lodish, 2003), led these researchers to propose that adiponectin and TNFα reciprocally regulate each other. In light of the emerging evidence that the marked increase in TNFα (and IL6) production in adipose tissue which accompanies obesity is in fact an inflammatory response (Black, 2003; Xu et al., 2003), adiponectin may have a particularly important metabolic role as a regulator of local cytokine production.

Adiponectin suppresses activation of the NFkB transcription factor, and induces PPARα and PPARγ expression. To date, there is little information available as to the signaling pathway used by adiponectin to suppress cytokine production. Using aortic endothelial cells as a model system, Ouchi et al. (2000) found that adiponectin caused an accumulation of cAMP and blocked TNFα signaling in these cells by preventing the activation and translocation of NFkB transcription factor to the nucleus. We have documented a similar action of adiponectin in pig macrophages (Wulster-Radcliffe et al., 2004), and in pig adipocytes (Ajuwon and Spurlock, 2004). Because NFkB is a major regulator of TNFα and IL6 expression (Vanden Berghe et al., 1999; Wajant et al., 2003), these findings have led us to hypothesize that the anti-inflammatory actions of adiponectin are mediated in part by inhibition of NFkB activation. This hypothesis is also supported by the indications that both PPARα and PPARγ are potent anti-inflammatory mediators which block NFkB activity to disrupt proinflammatory cytokine production (see Vanden Berghe et al., 2003 for a detailed description), and by evidence that adiponectin stimulates the expression of these transcription factors in adipocytes (Combs et al., 2004) or other cells (Yamauchi et al., 2001a;Yamauchi et al., 2003).

Implications for Pork Production

Our understanding of the central role of the adipocyte and its hormonal products in the regulation of energy metabolism is in its infancy. However, leptin and adiponectin have particular relevance to the energy metabolism in commercial pigs for several reasons, some of which clearly overlap. First, there has been a considerable effort in the past 2 decades to reduce carcass fat in commercial swine. With the success in this endeavor, there has emerged a consensus opinion among scientists that the perpetual exposure of animals in commercial production to pathogenic and nonpathogenic challenges results in a chronic stimulation of the immune system that ultimately diminishes growth and efficiency (Spurlock, 1997). Thus, there is a clear need to understand the impact of reduced carcass fat on adipocyte number and the regulation of adiponectin and leptin production, and to consider these effects in light of biological pathways regulated by these adipocyte-derived hormones. Pigs with higher concentrations of adiponectin may be less affected by the nonpathogenic challenge, but in their environment they may also have greater difficulty responding to more serious pathogenic challenges. Secondly, an allelic variant or genetic predisposition that either augments or attenuates the production and activity of leptin or adiponectin may result in aberrations in energy metabolism and the immune response that compromise efficiency or perhaps animal health. One documented missense mutation in humans was associated with markedly low concentrations of plasma adiponectin (Takahashi et al., 2000), and similar variants are possible across swine genotypes. Finally, a clear metabolic link between adipocytes and myofibers is indicated in that both leptin and adiponectin regulate metabolism in skeletal muscle. This further emphasizes the role of the adipocyte in energy homeostasis, and opens up a wealth of new opportunities for scientist to identify new pathways and targets for improving growth and efficiency in pigs reared commercially.

References

Ahima, R. S. and J. S. Flier. 2000. Leptin Annual Review of Physiology 62:413-437.

Ahima, R. S., D. Prabakaran, C. Mantzoros, D. Qu, B. B. Lowell, E. Maratosflier, and J. S. Flier. 1996. Role of leptin in the neuroendocrine response to fasting. Nature 382:250-252.

Ajuwon, K. M., J. L. Kuske, D. B. Anderson, D. L. Hancock, K. L. Houseknecht, O. Adeola, and M. Spurlock. 2003. Chronic leptin administration increases serum NEFA in the pig and differentially regulates PPAR expression in adipose tissue. J Nutr Biochem. 14:576-583.

Ajuwon, K. M., S. K. Jacobi, J. L. Kuske, and M. E. Spurlock. 2004. Interleukin-6 and interleukin-15 are selectively regulated by lipopolysaccharide and interferon-□ in primary pig adipocytes. Am J Physiol Regul Integr Comp Physiol. 286(3):R547-R553.

Ajuwon, K. M., M. E. Spurlock. 2004. Adiponectin inhibits NFkB activation, suppresses IL-6 production, and increases PPAR□2 expression in Adipocytes. Am J Physiol Regul Integr Comp Physiol. (Submitted for publication).

Arita, Y, S. Kihara, N. Ouchi, M. Takahashi, K. Maeda, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, H. Kuriyama, M. Nishida, S. Yamashita, K. Okubo, K. Matsubara, M. Muraguchi, Y. Ohmoto, T. Funahashi, Y. Matsuzawa. 1999. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 257(1):79-83.

Atkinson, L. L., M. A. Fischer and G. D. Lopaschuk. 2002. Leptin activates cardiac fatty acid oxidation independent of changes in the AMP-activated protein kinase-acetyl-CoA carboxylase-malonyl-CoA axis. J. Biol. Chem. 277:29424-29430.

Barb, C. R., G. J. Hausman and K. L. Houseknecht. 2001. Biology of leptin in the pig. Domest Anim. Endocrinol. 21:297-317.

Berg, A. H., T. P. Combs, X. Du, M. Brownlee, P. E. Scherer. 2001. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat. Med. 7(8):947-53.

Bidwell, C. A., S. Q. Ji, G. R. Frank, S. G. Cornelius, G. M. Willis, M. E. Spurlock. 1997. Cloning and expression of the porcine obese gene. Anim. Biotech. 8:191-206.

Black, P.H. 2003. The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. Brain Behav. Immun.17(5):350-64.

Chen, L., M. Anton, F. L. Graham. 1996. Production and characterization of human 293 cell lines expressing the site-specific recombinase Cre. Somat Cell Mol Genet. 22(6):477-88.

Chen, Y. and M. L. Heiman. 2000. Chronic leptin administration promotes lipid utilization until fat mass is greatly reduced and preserves lean mass of normal female rats. Regul. Pept. 92:113-119.

Chen, Y. and M. L. Heiman. 2001. Increased weight gain after ovariectomy is not a consequence of leptin resistance. AJP Endocrinology and Metabolism. 280:E315-E322.

Combs, T. P., U. B. Pajvani, A. H. Berg, Y. Lin, L. A. Jelicks, M. Laplante, A. R. Nawrocki, M. W. Rajala, A. F. Parlow, L. Cheeseboro, Y. Y. Ding, R. G. Russell, D. Lindemann, A. Hartley, G. R. Baker, S. Obici, Y. Deshaies, M. Ludgate, L. Rossetti, P. E. Scherer. 2004. A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. Endocrinology. 145(1):367-83.

Considine, R. V., R. C. Cooksey, L. B. Willims, R. L. Fawcett, P. L. Zhang, W. T. Ambrosius, R. M. Whitfield, R. Jones, M. Inman, J. Huse and D. A. McClain. 2000. Hexosamines regulate leptin production in human subcutaneous adipocytes. J. Clin. Endocrinol. Metabol. 85:3551-3556.

Doris, R. A., E. Kilgour, M. D. Houslay and R. G. Vernon. 1998. Regulation of the GTP-binding protein-based antilipolytic system of sheep adipocytes by growth hormone. J. Endocrinol. 58:295-303.

Finn, P. D., M. J. Cunningham, K. Y. Pau, H. G. Speis, D. K. Clifton and R. A. Steiner. 1998. The stimulatory effect of leptin on the neuroendocrine reproductive axis of the monkey. Endocrinology. 139:4652-4662.

Flier, J. S. 1998. Clinical review 94: What's in a name? In search of leptin's physiologic role. J. Clin. Endocrinol. Metabol. 83:1407-1413.

Friedman, J. M. and J. L. Halaas. 1998. Leptin and the regulation of body weight in mammals. Nature. 395:763-770.

Fruebis, J., T. S. Tsao, S. Javorschi, D. Ebbets-Reed, M. R. Erickson, F. T. Yen, B. E. Bihain, H. F. Lodish. 2001. Proteolytic cleavage product of 30-kDa Adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Aci U S A. 98(4):2005-10.

Frühbeck, G., M. Aguado and J. A. Martinez. 1997. In vitro lipolytic effect of leptin on mouse adipocytes: Evidence for a possible autocrine/paracrine role of leptin. Biochem. Biophys. Res. Comm. 240:590-594.

. Frühbeck, G., M. Aguado, J. Gomez-Ambrosi and J. A. Martinez. 1998. Lipolytic effect of in vivo leptin administration on adipocytes of lean and ob/ob mice, but not db/db mice. Biochem. Biophys. Res. Comm. 250:99-102.

Fruhbeck, G., J. Gomez-Ambrosi and J. Salvador. 2001. Leptin-induced lipolysis opposes the tonic inhibition of endogenous adenosine in white adipocytes. Faseb J. 15:333-340.

Higa, M., T. Kakuma, W. Pan, Z. W. Wang, E. Babcock, K. McCorkler, Y. Lee and R. Unger. 2000. Slow recovery of body fat lost during adenovirus-induced hyperleptinemia. Biochem. Biophys. Res. Comm. 279:786-791.

Houseknecht, K. L. and D. E. Bauman. 1997. Regulation of lipolysis by somatotropin: functional alteration of adrenergic and adenosine signaling in bovine adipose tissue. J. Endocrinol. 152:465-75.

Houseknecht, K. L. and C. P. Portocarrero. 1998. Leptin and its receptors: Regulators of whole-body energy homeostasis. Domest. Anim. Endocrinol. 15:457-475.

Hu, E., P. Liang and B. M. Spiegelman. 1996. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J. Biol. Chem. 271(18):10697-703

Jacobi, S., K. M. Ajuwon, T. E. Weber, J. L. Kuske, C. J. Dyer, and M. E. Spurlock. 2004. Cloning and expression of porcine adiponectin, and its relationship to adiposity, lipogenesis, and the acute phase response. J. Endo. 2004 (in press). ARP # 17192.

Lee, Y., M. Y. Wang, T. Kakuma, Z. W. Wang, E. Babcock, K. McCorkle, M. Higa, Y. T. Zhou and R. H. Unger. 2001. Liporegulation in diet-induced obesity. The antisteatotic role of hyperleptinemia. J. Biol. Chem. 276:5629-5635.

Lee, Y., X.Yu, F. Gonzales, D. J. Mangelsdorf, M. Y. Wang, C. Richardson, L. A. Witters and R. H. Unger. 2002. PPAR alpha is necessary for the lipopenic action of hyperleptinemia on white adipose and liver tissue. Proceedings of the National Academy of Sciences, USA 99:11848-11853.

Legradi, G., C. H. Emberson, R. S. Ahima, J. S. Flier and R. M. Lechan. 1997. Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleaic acid in neurons of the hypothalamic paraventricular nucleus. Endocrinology. 138:2569-2576.

Lin, S., L. H. Storlein and X. Huang. 2000. Leptin receptor, NPY, POMC mRNA expression in the diet-induced obese mouse brain. Brian Res. 875:89-95.

Lõllmann, B., S. Gruninger, A. Stricker-Krongrad and M. Chiesi. 1997. Detection and quantification of the leptin receptor splice variants Ob-Ra, b, and e in different mouse tissues. Biochem. Biophys. Res. Comm. 238:648-652.

Lord, G. M., G. Matarese, J. K. Howard, R. J. Baker, S. R. Bloom and R. I. Lechler. 1998. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature. 294:897-891.

Madej, T., M. S. Boguski and S. H. Bryant. 1995. Threading analysis suggests that the obese gene product may be a helical cytokine. FEBS Lett. 373:13-18.

Maeda, K., K. Okubo, I. Shimomura, T. Funahashi, Y. Matsuzawa, K. Matsubara. 1996.

cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem. Biophys. Res. Comm. 221(2):286-9.

Marshall, S., W. T. Garvey and R. R. Traxinger. 1991. New insights into the metabolic regulation of insulin action and insulin resistance: role of glucose and amino acids. Faseb J. 5:3031-3036.

McClain, D. A., T. Alexander, R. C. Cooksey and R. V. Considine. 2000. Hexosamines stimulate leptin production in transgenic mice. Endocrinology. 141:1999-2002.

Minokoshi, Y., Y. B. Kim, O. D. Peroni, L. G. Fryer, C. Muller, D. Carling and B. B. Kahn. 2002. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature. 17:339-343.

Mueller, W., F. M. Gregoire, K. Stanhope, C. V. Mobbs, T. M. Mizuno, C. Warden, J. S. Stern and P. J. Havel. 1998. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology. 139:551-558.

Müller, G., J. Ertl, M. Gerl, and G. Preibisch. 1997. Leptin impairs metabolic actions of insulin in isolated rat Adipocytes. Am. Soc. Biochem. Mol. Biol. 272(15):10585-10593.

Muoio, D. M., F. T. Fiedorek Jr., E. B. Taspscot, R. A. Coleman, and G. L. Dohm. 1997. Leptin directly alters lipid partitioning in skeletal muscle. Diabetes. 46:1360-1363.

Muoio, D. M., G. L. Dohm, E. B. Tapscott and R. A. Coleman. 1999. Leptin opposes insulin's effect on fatty acid partitioning in muscles isolated from obese ob/ob mice. Am. J. Physiol. 276:E913-E921.

Nagatani, S., P. Guthikonda, R. C. Thompson, H. Tsukamura, K. I. Maeda, and D. L. Foster. 1998. Evidence for GnRH regulation by leptin: Leptin administration prevents reduced pulsatile LH secretion during fasting. Neuroendocrinology. 67:370-376.

Obici, S., Z. H. Feng, Y. Morgan, D. Stein, G. Karkanias and L. Rossetti. 2002a. Central administration of oleic acid inhibits glucose production and food intake. Diabetes. 51:271-275.

Obici, S., J. Wang, R. Chowdury, Z. Feng, U. Siddhanta, K. Morgan and L. Rossetti. 2002b. Identification of a biochemical link between energy intake and energy expenditure. J. Clin. Invest. 109:1599-1605.

Ouchi, N., S. Kihara, Y. Arita, Y. Okamoto, K. Maeda, H. Kuriyama, K. Hotta, M. Nishida, M. Takahashi, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Funahashi, Y. Matsuzawa. 2000. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation. 102(11):1296-301.

Qian, H., D. L. Hartzell, C. A. Baile, M. J. Azain, M. M. Compton and G. J. Hausman. 1998b. Brain administration of leptin causes deletion of adipocytes by apoptosis. Endocrinology. 139:791-794.

Ramsay, T. G. 1996. Fat cells. Endocrinology & Metabolism Clinics of North America 25:847-70.

Ramsay, T. G. 2001. Porcine leptin alters insulin inhibition of lipolysis in porcine adipocytes in vitro. J. Anim. Sci. 79:653-657.

Reidy, S. P. and J. M. Weber. 2002. Accelerated substrate cycling: a new energy-wasting role for leptin in vivo. AJP Endocrinology and Metabolism. 282:E312-E317.

Ruan, H., H. F. Lodish. 2003. Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-alpha. Cytokine Growth Factor Rev. 14(5):447-55.

Ruderman, N. B., H. Park, V. K. Kaushik, D. Dean, S. Constant, M. Prentki, A. K. Saha. 2003. AMPK as a metabolic switch in rat muscle, liver and adipose tissue after exercise. Acta. Physiol. Scand. 178(4):435-42.

Sarmiento, U., B. Benson, S. Kaufman, L. Ross, M. Qi , S. Scully and C. DiPalma. 1997. Morphologic and molecular changes induced by recombinant human leptin in the white and brown adipose tissues of C57BL/6 mice. Lab. Investig. 77:243-256.

Scarpace, P. J., M. Nicolson and M. Matheny. 1998. UCP1, UCP3 and leptin gene expression: modulation by food restriction and leptin. J. Endocrinol. 159:349-357.

Scherer, P. E., S. Williams, M. Fogliano, G. Baldini, H. F. Lodish. 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. J. Biol. Chem. 270(45):26746-9.

Scherer, P. E., T. Okamoto, M. Chun, I. Nishimoto, H. F. Lodish, and M. P. Lisanti. 1996. Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. Proc. Natl. Acad. Sci., USA. 93(1):131-5.

Sethi, J. K. and G. S. Hotamisligil. 1999. The role of TNF alpha in adipocyte metabolism. Semin. Cell. Dev. Biol. 10(1):19-29. Review

Shimabukuro, M., K. Koyama, Y. Lee and R. H. Unger. 1997. Leptin- or troglitazone- induced lipopenia protects islets from interleukin 1 beta cytotoxicity. J. Clin. Investig. 100:1750-1704.

Slieker, L. J., K. W. Sloop, P. L. Surface, A. Kriauciunas, F. LaQuier, J. Manetta, J. Bue-Valleskey and T. W. Stephens. 1996. Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. J. Biol. Chem. 271:5301-5304.

Spurlock, M. E. 1997. Regulation of metabolism and growth during immune challenge: an overview of cytokine function. J. Anim Sci. 75(7):1773-83.

Steinberg, G. R., M. L. Parolin, G. J. Heigenhauser and D. J. Dyck. 2002a. Leptin increases fatty acid oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. AJP Endocrinology and Metabolism. 283:E187-E192.

Steinberg, G. R., A. Bonen and D. J. Dyck. 2002b. Fatty acid oxidation and triacylglycerol hydrolysis are enhanced after chronic leptin treatment in rats. AJP Endocrinology and Metabolism. 282: E593-E600.

Steinberg, G. R., D. J. Dyck, J. Calles-Escandon, N. N. Tandon, J. J. Luiken, J. F. Glatz and A. Bonen. 2002c. Chronic leptin administration decreases fatty acid uptake and fatty acid transporters in rat skeletal muscle. J. Biol. Chem. 277:8854-8860.

Sweeney, G. 2002. Leptin signaling. Cell. Signal. 14:655-663.

Takahashi, Y. and T. Ide. 2000. Dietary n-3 fatty acids affect mRNA level of brown adipose tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat. Brit. J. Nutr. 84:175-184.

Tartaglia, L., M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Deros, G. J. Richards, L. A. Camfield, F. T. Clark, J. Deeds, C. Muri, S. Sanker, A. Moriarty, K. J. Moore, J. S. Smutoko, G. G. Mays, E. A. Woolf, C. A. Monroe and R. I. Tepper. 1995. Identification and expression cloning of a leptin receptor. Cell. 83:1263-1271.

Tomas, E., T. S. Tsao, A. K. Saha, H. E. Murrey, Cc. C. Zhang, S. I. Itani, H. F. Lodish, N. B. Ruderman. 2002. Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc. Natl. Acad. Sci., USA. 99(25):16309-13.

Vanden Berghe, W., K. De Bosscher, E. Boone, S. Plaisance, G. Haegeman. 1999. The nuclear factor-kappa-B engages CBP/p300 and histone acetyltransferase activity for transcriptional activation of the interleukin-6 gene promoter. J. Biol. Chem. 274(45):32091-8.

Wajant, H., K. Pfizenmaier, and P. Scheurich. 2003. Tumor necrosis factor signaling. Cell Death Differ. 10(1):45-65. Review.

Wang, J., R. Liu, M. Hawkins, N. Barzilai and L. Rossetti. 1998. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature. 393:684-688.

Wang, M., Y. Lee and R. H. Unger. 1999a. Novel form of lipolysis induced by leptin. J. Biol. Chem. 274:17541-17544.

Wang, J., R. Liu, L. Liu, R. Chowdhury, N. Barzilai, J. Tan and L. Rossetti. 1999b. The effects of leptin on Lep expression is tissue-specific and nutritionally regulated. Nat. Med. 5:895-899.

Wulster-Radcliffe, M. C., K. M. Ajuwon, J. Wang, J. A. Christian, and M. Spurlock. 2004. Adiponectin differentially regulates pro- and anti-inflammatory cytokine in porcine macrophages. Biochem. Biophys. Res. Comm. 316:924-929.

Xu, H., G. T. Barnes, Q. Yang, G. Tan, D. Yang, C. J. Chou, J. Sole, A. Nichols, J. S. Ross, L. A. Tartaglia, and H. Chen. 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest. 112(12):1821-30.

Yamauchi, T., J. Kamon, H. Waki, K. Murakami, K. Motojima, K. Komeda, T. Ide, N. Kubota, Y. Terauchi, K. Tobe, H. Miki, A. Tsuchida, Y. Akanuma, R. Nagai, S. Kimura, and T. Kadowaki. 2001a. The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance. J. Biol. Chem. 276(44):41245-54

Yamauchi, T., J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B. B. Kahn, and T. Kadowaki. 2002. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat. Med. 8(11):1288-95.

Yamauchi, T., J. Kamon, Y. Ito, A. Tsuchida, T. Yokomizo, S. Kita, T. Sugiyama, M. Miyagishi, K. Hara, M. Tsunoda, K. Murakami, T. Ohteki, S. Uchida, S. Takekawa, H. Waki, N. H. Tsuno, Y. Shibata, Y. Terauchi, P. Froguel, K. Tobe, S. Koyasu, K. Taira, T. Kitamura, T. Shimizu, R. Nagai, and T. Kadowaki. 2003. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature. 423(6941):762-9.

Yoda, M., Y. Nakano, T. Tobe, S. Shioda, N. H. Choi-Miura, and M. Tomita. 2001. Characterization of mouse GBP28 and its induction by exposure to cold. Int. J. Obes. Relat. Metab. Disord. 25(1):75-83.

Yokota, T., K. Oritani, I. Takahashi, J. Ishikawa, A. Matsuyama, N. Ouchi, S. Kihara, T. Funahashi, A. J. Tenner, Y. Tomiyama, and Y. Matsuzawa. 2000. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood. 96(5):1723-32.

Yu, W. H., M. Kimura, A. Walczewska, S. Karanth and S. M. McCann. 1997. Role of leptin in hypothalamic-pituitary function. Proc. Natl. Acad. Sci., USA. 94:1023-1028.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425-432.

Zhang, P. L., E. S. Klenk, M. A. Lazzaro, L. B. Williams and R. V. Considine. 2002. Hexosamines regulate leptin production in 3T3-L1 adipocytes

through transcriptional mechanisms. Endocrinology. 143:99-106.

Zhou, T. Y., Z. W. Wang, M. Higa, C. B. Newgard and R. H. Unger. 1999. Reversing adipocyte differentiation: implications for treatment of obesity. Proc. Natl. Acad. Sci., USA. 96:2391-2395.

Zurlo, F., K. Larson, C. Bogardus and E. Ravussin. 1990. Skeletal muscle metabolism is a major determinant of resting energy expenditure. J. Clin. Invest. 86:1423-1427.

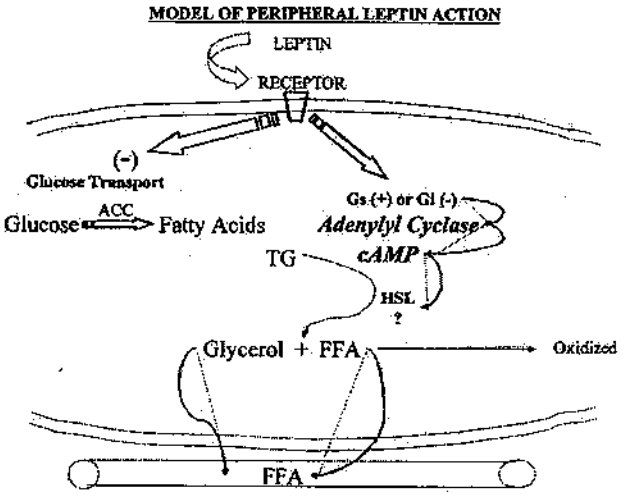


Figure 1. Leptin targets acetyl-CoA carboxylase (ACC) expression and activity to decrease lipogenesis, and disrupts glucose uptake. Triglyceride (TG) lipolysis is activated via an inhibition of the inhibitory G protein (Gi) or a stimulation of the stimulatory G protein (Gs). The production of cAMP is increased and hormone sensitive lipase (HSL) activated. Glycerol is released from the cell, whereas fatty acids (FFA) may be released or oxidized internally.

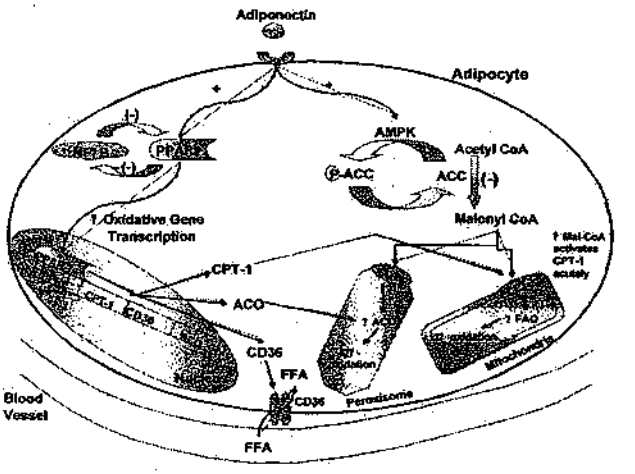


Figure 2. Schematic illustrating a potential mechanism by which adiponectin diminishes lipogenesis, and promotes fatty acid oxidation rather than storage. Adiponectin stimulates the 5'-AMP-activated protein kinase (AMPK), which causes phosphorylation and deactivation of acetyl Co-A-carboxylase (ACC), the rate limiting enzyme for lipogenesis. Malonyl Co-A is depleted and its inhibition of carnitine plamitoyl transferase (CPT-1) and carnitine octanoyl transferase (COT) is relieved, thus allowing greater fatty acid transport into the mitochondria and peroxisomes, respectively, for oxidation. Additionally, adiponectin stimulates the expression of PPAR□, a transcription factor which stimulates expression of acyl Co-A oxidase (ACO), the mitochondrial fatty acid oxidase complex (FAO), a fatty acid transport protein (CD36), and COT and CPT-1, which, collectively, enhance the oxidative capabilities of the cell.

Nutritional Effects on Disease Resistance

Rodney W. Johnson
Department of Animal Sciences
University of Illinois
Urbana, IL 61801
217-333-2118
rwjohn@uiuc.edu

Jeffery Escobar
USDA/ARS Children's Nutrition Research Center
Department of Pediatrics-Nutrition
Baylor College of Medicine
1100 Bates St Room 9022
Houston, TX 77030
713-798-7051
jescobar@bcm.tmc.edu

Summary

The NRC nutrient requirements for optimal productivity are well defined. However, because the NRC requirements have been established from studies where infectious disease is minimal, it is not clear if the nutrient requirements for optimal productivity confer optimal immunity in the presence of infectious disease. The requirement for a given nutrient that has immunomodulatory properties may vary depending upon the presence or absence of infectious disease. The purpose of this brief paper is to review several nutrients that have immunomodulatory properties.

Introduction

Nutrient requirements for animals established by the National Research Council (NRC) (NRC, 1998) can be defined as levels adequate to permit the maintenance of normal health and productivity. Failure to provide a diet that does not fulfill the minimal requirements established by NRC for any nutrient will ultimately immunocompromise the animal and render it more susceptible to infectious disease. Because nutrient requirements to support optimal productivity are well defined, marked deficiencies in protein, amino acids, or trace nutrients, are not likely to occur in animals reared in commercial situations. However, the nutrient requirements for optimal productivity may not equal those for optimal immunity because the NRC requirements have been determined from experiments conducted in laboratory situations where infectious disease is minimal. Thus, an important issue that has been the focus of nutritional immunology research is whether specific nutrients fed at or above NRC recommended levels could be used to modulate the animal's immune system in a beneficial manner.

The Immune System

Animals live surrounded by pathogenic microorganisms that can cause infectious disease or pathology. However, they fall ill relatively infrequently because they are equipped with a well-evolved immune system that affords protection against foreign agents. The cells of the immune system and their responses to infection are obviously complex but can, in general, be partitioned into two separate but interacting components—that which provides innate immunity and that which provides acquired (or adaptive) immunity (**Figure 1**). Both components are influenced by nutrition (**Table 1**).

The component of the immune system that protects the host animal but does not distinguish one pathogen from another provides innate immunity. For example, macrophages recognize pathogens using relatively indiscriminant and invariant receptors. They ingest and degrade microorganisms, and provide important signals (e.g., inflammatory cytokines) that orchestrate other aspects of the immune response. The innate immune system is inherent to the individual and the capacity of it to respond does

not change or improve from the first encounter with a particular pathogen to the second encounter. Neutrophils and natural killer (NK) cells are also important for innate immunity.

Acquired immunity is a highly specific response to a specific pathogen that is acquired over time due to previous exposure to that same pathogen or through vaccination. Fully differentiated B lymphocytes (i.e., plasma cells) secrete pathogen-specific antibodies, whereas T lymphocytes use discrete receptors to recognize and kill infected cells or activate other cells of the immune system. The initial exposure to a pathogen produces a population of lymphocytes with immunological memory so that if the pathogen is encountered a second time, a rapid response is initiated and the pathogen is eliminated before visible signs of infection appear.

Immunomodulatory Effects of Nutrients

Amino Acids

Although numerous amino acids have important roles in the proper functioning of the immune system, methionine, arginine, and glutamine seem to be the ones that are required in the greatest quantities during an immune response. Methionine is the first limiting amino acid in most practical poultry diets, and it is the second or third limiting amino acid in barley and wheat-based swine diets, making it a primary concern for marginal deficiency. Tsiagbe et al. (Tsiagbe et al., 1987) showed that chicks required a greater quantity of methionine to maximize immune responses to sheep red blood cells and delayed-type hypersensitivity to phytohemagglutinin, but other investigators have not observed this relationship (Bhargva et al., 1970), so the idea that animals may require methionine for immune function at levels above those that support maximal growth is not entirely agreed upon.

Arginine is considered a semi-essential amino acid for humans and other mammals because it is synthesized from other amino acids via the urea cycle. However, exogenous arginine is required for growth in young animals and in various stress situations (e.g., sepsis, trauma) to optimize growth and minimize nitrogen excretion (Barbul and Dawson, 1994). The finding that arginine is a direct precursor of nitric oxide (NO), a potent cytotoxic agent produced by macrophages and neutrophils to kill bacteria, highlights the importance of arginine to immune function.

Although glutamine is not considered to be an indispensable amino acid for growth of animals, recent research has indicated that it may be conditionally essential in times of immune system activation (Newsholme et al., 1988; Lacey and Wilmore, 1990). This hypothesis stemmed from research showing that glutamine is essential for the normal functioning of macrophages and lymphocytes during an immune response (Lacey and Wilmore, 1990; Dudrick et al., 1994). The requirement for glutamine in these cells is due to the increased metabolic activity following stimulation by an infectious pathogen. The accelerated metabolism is necessary to facilitate cell division and the secretion of antibodies and cytokines, all processes that require amino acids and energy. Glutamine is a primary carrier of nitrogen in the blood, and its concentration is generally maintained within a relatively small range. However, during catabolic states like sepsis, there is an increased demand for glutamine as a substrate for cells of the immune system.

Lipids

Feeding high-fat diets reduces lymphocyte proliferation, when compared to low-fat diets, but the precise effects depend on the amount and type of dietary fat (Calder, 1998; Calder and Grimble, 2002). There are two major classes of polyunsaturated fatty acids (PUFAs)—the n-6 and the n-3 families. Linoleic acid is the precursor of the n-6 family, and it is found in plant oils, including corn and soybean oil. In animals, linoleic acid is converted to arachidonic acid, which can account for 25% of the total fatty acids in the plasma membranes of immune cells. The amount of arachidonic acid in the plasma membrane of immune cells is important because it is the precursor of several prostaglandins and leukotrienes that have potent inflammatory effects. The precursor of the n-3 PUFAs is α-linolenic acid, which in animal tissues is converted to eicosapentaenoic and docosahexaenoic acids. As opposed to n-6 PUFAs that are inflammatory, n-3 PUFAs are anti-inflammatory.

Compared with diets rich in n-6 PUFAs, feeding rodents diets rich in fish oil (i.e., diets rich in eicosapentaenoic and docosahexaenoic acids) results in suppressed proliferation of T- and B-lymphocytes, decreased IL-2 (a cytokine that causes T-cell proliferation) production by lymphocytes, decreased T-lymphocyte cytotoxicity, decreased NK cell activity, and decreased macrophage-mediated cytolysis (Anderson and Fritsche, 2002). Consistent with studies in

rodents, in pigs, dietary fish oil decreased leukocyte phagocytosis, lymphocyte proliferation, and NK cell activity (Thies et al., 1999). The production of inflammatory cytokines by monocytes is also inhibited by n-3 PUFAs.

Diets rich in n-3 PUFAs decrease inflammation at least two ways. First, consumption of a diet rich in n-3 PUFAs increases membrane levels of eicosapentaenoic and docosahexaenoic acids at the expense of arachidonic acid. Thus, when immune cells are stimulated, there is less arachidonic acid available to generate prostaglandins and leukotrienes, which are inflammatory in nature (Fritsche et al., 1993; Thies et al., 1999). Second, eicosapentaenoic acid is a substrate for the same enzymes that metabolize arachidonic acid. However, the products of eicosapentaenoic acid metabolism are less potent inflammatory molecules than are those generated by metabolism of arachidonic acid.

Although it might be useful to consume high levels of n-3 PUFAs to decrease inflammation associated with autoimmune and neoplastic disease, or reduce the risk of heart disease, these conditions are not especially relevant to food animal production, and the immunosuppression may render animals more susceptible to infectious disease. Indeed, several studies have shown that dietary fish oil can increase the susceptibility of animals to some bacterial infections (Chang et al., 1992; Fritsche et al., 1997; Irons et al., 2003). Thus, inclusion of fish or other n-3 PUFA-rich oils in animal diets should be approached with caution to avoid increased incidence of infections.

Zinc

Zinc (Zn) is a component of at least 300 enzymes, and inadequate intake of Zn renders animals severely immunodeficient and highly susceptible to viral, bacterial, and parasitic microorganisms (Shankar and Prasad, 1998). Both innate and acquired immunity are affected (Dardenne, 2002; Ibs and Rink, 2003). Thymulin is a hormone that promotes T-cell development and function (Pleau et al., 1980). For activation, thymulin must undergo a conformational change, which is induced by binding Zn. Therefore, in Zn-deficient animals even though thymulin is detectable in serum, it is inactive and the utility of T-cells is severely diminished. Interestingly, pigs nursed by sows exposed to aflatoxins have symptoms suggesting Zn deficiency, including growth retardation, thymic involution, and impaired immunocompetence. Thymulin in pigs exposed to maternal aflatoxins was inactive, but could be activated *in vitro* by addition of physiologically relevant levels of Zn (Mocchegiani et al., 1998).

Some studies suggest that the Zn required for optimum immunity is higher than that for optimum productivity. During the acute-phase response Zn is redistributed from the plasma to the liver and to lymphocytes. In humans, daily Zn supplementation reduced the incidence and duration of diarrhea and reduced the incidence of acute and lower respiratory infections (Sazawal et al., 1996; Sazawal et al., 1998). Strains of mice that were genetically susceptible to infection by *Candida albicans* became resistant when fed a Zn-enriched diet or when injected intraperitoneally with Zn, whereas normally resistant mice became susceptible when fed diets inadequate in Zn (Salvin et al., 1987). Adverse effects of Zn excess on lymphocyte proliferation and chemotaxis and phagocytosis of neutrophils has been reported (Chandra, 1984), so caution must be exercised when using Zn as an immunomodulator.

Iron and Copper

The effect of iron (Fe) on immunocompetence is not as clear as that of Zn; however, generally speaking an imbalance in Fe intake—either too much or too little—decreases immunity. One of the acute responses induced by infection is hypoferremia. The inflammatory cytokines released by activated macrophages cause Fe to be sequestered. Because Fe is a rate-limiting nutrient for the growth of several pathogenic microorganisms, its removal from blood and temporary storage in compartments that are not accessible to pathogens is considered part of the host defense. Iron-binding proteins chelate most Fe; however, supplementation can saturate these proteins, leaving excess Fe available to pathogens. For instance, pigs that were given 100 mg Fe dextran and then inoculated with *Escherichia coli* (*E. coli*) into ligated intestines demonstrated increased total Fe-binding capacity, increased liver Fe content and decreased plasma Fe concentration (Knight et al., 1984). However, pigs that received 400 mg Fe dextran prior to inoculation with *E. coli* had increased Fe in liver, but they were not able to increase total Fe binding capacity and thus could not limit the availability of Fe to the pathogenic microorganism (Knight et al., 1984). Thus, even though supplemental Fe is needed to prevent anemia in newborn pigs, excess Fe can actually enhance the growth of certain pathogenic microorganisms.

Copper (Cu) status is determined primarily by the plasma concentration of the acute-phase protein ceruloplasmin. The inflammatory cytokines induce synthesis of ceruloplasmin. Therefore, whereas infection decreases circulating Fe, it increases circulating Cu. The increase in plasma Cu may be to enhance lymphocyte responses. Splenocytes isolated from Cu-deficient rats produced less IL-2 when compared with splenocytes from controls (Hopkins and Failla, 1995; Percival, 1998). Interleukin-2 is a cytokine produced by T-cells that acts in an autocrine manner to promote proliferation. Accordingly, the proliferation of splenocytes from Cu-deficient rats also decreased when compared with control splenocytes. To our best knowledge, there have been no studies to determine if the Cu required for optimum immunity is higher than that for optimum production.

Vitamin E and Selenium

The primary role of vitamin E in nutrition is to protect cellular membranes from peroxidative damage (Sheffy and Schultz, 1979), whereas Se is an integral component of glutathione peroxidase. Numerous studies have suggested that vitamin E and Se also play an active role in the host's response to infection (Meydani and Beharka, 1998). Lessard et al. (Lessard et al., 1991) fed pigs a diet adequate in vitamin E and Se or a diet deficient in both for 21 d before they were inoculated with *Salmonella typhisuis* (*S. typhisuis*). Whereas vitamin E and Se deficiency did not influence the proliferation of lymphocytes from unchallenged pigs, it markedly suppressed the proliferation of lymphocytes that were obtained from pigs that had been inoculated with *S. typhisuis*.

Vitamin E and Se supplementation in excess of minimal requirements has been shown to increase antibody production and lymphocyte proliferation in pigs (Ellis and Vorhies, 1976; Mahan and Moxon, 1980; Peplowski et al., 1980; Larsen and Tollersrud, 1981). Vitamin E supplementation enhances the random migration, chemotaxis, and phagocytic activity of mouse peritoneal macrophages (Del Rio et al., 1998) and has been shown to protect mice from influenza infection (Hayek et al., 1997; Han et al., 2000). However, in a recent study feeding a vitamin E level 50 times the NRC requirement did not afford pigs protection from the effects of PRRSV infection on growth performance, cytokine production, and certain hematological traits (e.g., white blood cell counts) (Toepfer-Berg et al., submitted). Other studies indicate that vitamin E reduces the production of certain inflammatory cytokines (Webel et al., 1998; Leshchinsky and Klasing, 2003; Godbout et al., 2004) and inhibits behavioral signs of sickness (Berg et al., 2004). Thus, in certain instances vitamin E supplementation may be beneficial: It appears that the reactive oxygen species generated during the immune response activate certain intracellular signaling pathways that regulate cytokine genes. Therefore, one way to regulate cytokines might be by use of antioxidants like vitamin E. More research is needed to clarify this point, however.

Vitamin A

Vitamin A deficiency severely compromises the integrity of mucosal epithelial cells in the respiratory, gastrointestinal, and uterine tracts. In the respiratory tract, ciliated columnar epithelium with mucus and goblet cells trap and remove inhaled microorganisms. In animals deficient in vitamin A, ciliated epithelial cells are replaced by stratified, keratinized epithelium, and there is a decrease in mucin. Similarly, in the small intestine, vitamin A deficiency results in a loss of microvilli, goblet cells, and mucin (De Luca et al., 1969; Rojanapo et al., 1980). Other effects of vitamin A deficiency on innate immunity include changes in epidermal keratins that disrupt skin barrier function (Molloy and Laskin, 1985); defects in chemotaxis, adhesion, phagocytosis, and the ability to produce reactive oxygen species in neutrophils (Twining et al., 1997); decreased number of NK cells and cytotoxicity (Zhao et al., 1994; Zhao and Ross, 1995); and a decrease in the expression of the receptor that recognizes gram negative bacteria as well as the secretion of inflammatory cytokines by macrophages and monocytes (Semba, 1998).

An adequate level of vitamin A is also necessary to support acquired immunity. The growth and activation of B cells requires retinol. Pigs deficient in vitamin A synthesize less than one tenth of the amount of antibody produced by pigs fed vitamin A-fortified diets (Harmon et al., 1963). In animals deficient in vitamin A, infection with *Trichinella spiralisa*, which normally induces strong T helper type 2-like responses (i.e., high levels of parasite-specific IgG and production of IL-4, IL-5, and IL-10), induced inappropriate strong T helper type 1-like responses (i.e., production of interferon-γ and IL-12) (Carman et al., 1992; Cantorna et al., 1994; Cantorna et al., 1995). In T-lymphocytes, retinoids increased expression of IL-2 receptors (Sidell et al., 1993) and increased antigen-specific proliferation (Friedman et al., 1993).

In chickens infected with Newcastle disease virus, vitamin A deficiency impaired T lymphocyte cytotoxicity (Sijtsma et al., 1990).

Nutrient Requirements for Optimal Immunity?

This paper has focused on how nutrition affects the immune system, but it must be noted that the immune system is not a passive participant in the interplay. When immune cells encounter a pathogen the cytokines that are released to orchestrate the immune response also act on other physiological systems that affect nutrient intake and utilization. Thus, the immune system can influence the nutrients required for optimal performance, and specific nutrients can either enhance or inhibit the immune system. A better understanding of this interaction is required, but several pragmatic applications of nutrient-immune interaction are already in sight. First, that infectious pathogens change animals' physiological state and alter nutrient requirements is inevitable. If these alterations in nutrient requirements can be precisely defined, it will be possible to formulate cost-effective diets that maximize productivity even if that productivity is less than it would have been had the animal not been infected. Second, it might be possible to develop diets that promote "optimal" immune responses. What is considered optimal may change from one production system to another, or even within a system depending on the disease environment at a given time. The goal need not always be to minimize the immune response, for in certain environments this might result in increased incidence of infection. Similarly, the goal need not always be to maximize the immune response because an over zealous response to nonpathogenic stimuli can be counterproductive. Finally, it is important to recognize that the physiological state of animals is very different prior to disease, during disease, and while recovering from disease. Therefore, the nutrient requirements of the immune system and growth during these three critical stages are likely to be different as well. Defining these presents an important challenge for the nutritional immunologist.

References

Anderson, M. and K. L. Fritsche. 2002. (n-3) Fatty acids and infectious disease resistance. J Nutr 132:3566-3576.

Barbul, A. and H. Dawson. 1994. Arginine and immunity. In: A. Forse (eds). In Diet, Nutrition and Immunityed. CRC Press, Inc. Boca Raton: 199.

Berg, B. M., J. P. Godbout, K. W. Kelley and R. W. Johnson. 2004. Alpha-tocopherol attenuates lipopolysaccharide-induced sickness behavior in mice. Brain Behav Immun 18:149-157.

Bhargva, K. K., R. P. Hanson and M. L. Sunde. 1970. Effects of methionine and valine on antibody production in chicks infected with Newcastle disease virus. J. Nutr. 100:241.

Calder, P. C. 1998. Fat chance of immunomodulation. Immunol Today 19:244-247.

Calder, P. C. and R. F. Grimble. 2002. Polyunsaturated fatty acids, inflammation and immunity. Eur J Clin Nutr 56 Suppl 3:S14-19.

Cantorna, M. T., F. E. Nashold and C. E. Hayes. 1994. In vitamin A deficiency multiple mechanisms establish a regulatory T helper cell imbalance with excess Th1 and insufficient Th2 function. J Immunol 152:1515-1522.

Cantorna, M. T., F. E. Nashold and C. E. Hayes. 1995. Vitamin A deficiency results in a priming environment conducive for Th1 cell development. Eur J Immunol 25:1673-1679.

Carman, J. A., L. Pond, F. Nashold, D. L. Wassom and C. E. Hayes. 1992. Immunity to Trichinella spiralis infection in vitamin A-deficient mice. J Exp Med 175:111-120.

Chandra, R. K. 1984. Excessive intake of zinc impairs immune responses. Jama 252:1443-1446.

Chang, H. R., A. G. Dulloo, I. R. Vladoianu, P. F. Piguet, D. Arsenijevic, L. Girardier and J. C. Pechere. 1992. Fish oil decreases natural resistance of mice to infection with Salmonella typhimurium. Metabolism 41:1-2.

Dardenne, M. 2002. Zinc and immune function. Eur J Clin Nutr 56 Suppl 3:S20-23.

De Luca, L., E. P. Little and G. Wolf. 1969. Vitamin A and protein synthesis by rat intestinal mucosa. J Biol Chem 244:701-708.

Del Rio, M., G. Ruedas, S. Medina, V. M. Victor and M. De la Fuente. 1998. Improvement by several antioxidants of macrophage function in vitro. Life Sci 63:871-881.

Dudrick, P. S., J. C. Alverdy and W. W. Souba. 1994. Glutamine and the immune system. In: A. Forse (eds). Diet, Nutrition and Immunityed. CRC Press, Inc. Boca Raton: 217.

Ellis, R. P. and M. W. Vorhies. 1976. Effect of supplemental dietary vitamin E on the serologic response of swine to an Escherichia coli bacterin. J Am Vet Med Assoc 168:231-232.

Friedman, A., O. Halevy, M. Schrift, Y. Arazi and D. Sklan. 1993. Retinoic acid promotes proliferation and induces expression of retinoic acid receptor-alpha gene in murine T lymphocytes. Cell Immunol 152:240-248.

Fritsche, K. L., S. C. Huang and M. Misfeldt. 1993. Fish oil and immune function. Nutr Rev 51:24.

Fritsche, K. L., L. M. Shahbazian, C. Feng and J. N. Berg. 1997. Dietary fish oil reduces survival and impairs bacterial clearance in C3H/Hen mice challenged with Listeria monocytogenes. Clin Sci (Lond) 92:95-101.

Godbout, J. P., B. M. Berg, K. W. Kelley and R. W. Johnson. 2004. alpha-Tocopherol reduces lipopolysaccharide-induced peroxide radical formation and interleukin-6 secretion in primary murine microglia and in brain. J Neuroimmunol 149:101-109.

Han, S. N., D. Wu, W. K. Ha, A. Beharka, D. E. Smith, B. S. Bender and S. N. Meydani. 2000. Vitamin E supplementation increases T helper 1 cytokine production in old mice infected with influenza virus. Immunology 100:487-493.

Harmon, B. G., E. R. Miller, J. A. Hoefer, D. E. Ullrey and R. W. Luecke. 1963. Relationships of specific nutrient deficiencies to antibody production in swine. I. Vitamin A. J. Nutr. 79:263.

Hayek, M. G., S. F. Taylor, B. S. Bender, S. N. Han, M. Meydani, D. E. Smith, S. Eghtesada and S. N. Meydani. 1997. Vitamin E supplementation decreases lung virus titers in mice infected with influenza. J Infect Dis 176:273-276.

Hopkins, R. G. and M. L. Failla. 1995. Chronic intake of a marginally low copper diet impairs in vitro activities of lymphocytes and neutrophils from male rats despite minimal impact on conventional indicators of copper status. J Nutr 125:2658-2668.

Ibs, K. H. and L. Rink. 2003. Zinc-altered immune function. J Nutr 133:1452S-1456S.

Irons, R., M. J. Anderson, M. Zhang and K. L. Fritsche. 2003. Dietary fish oil impairs primary host resistance against Listeria monocytogenes more than the immunological memory response. J Nutr 133:1163-1169.

Knight, C. D., K. C. Klasing and D. M. Forsyth. 1984. The effects of intestinal Escherichia coli 263, intravenous infusion of Escherichia coli 263 culture filtrate and iron dextran supplementation on iron metabolism in the young pig. J Anim Sci 59:1519-1528.

Lacey, J. M. and D. W. Wilmore. 1990. Is glutamine a conditionally essential amino acid? Nutr Rev 48:297-309.

Larsen, H. J. and S. Tollersrud. 1981. Effect of dietary vitamin E and selenium on the phytohaemagglutinin response of pig lymphocytes. Res Vet Sci 31:301-305.

Leshchinsky, T. V. and K. C. Klasing. 2003. Profile of chicken cytokines induced by lipopolysaccharide is modulated by dietary alpha-tocopheryl acetate. Poult Sci 82:1266-1273.

Lessard, M., W. C. Yang, G. S. Elliott, A. H. Rebar, J. F. Van Vleet, N. Deslauriers, G. J. Brisson and R. D. Schultz. 1991. Cellular immune responses in pigs fed a vitamin E- and selenium-deficient diet. J Anim Sci 69:1575-1582.

Mahan, D. C. and A. L. Moxon. 1980. Effects of dietary selenium and injectable vitamin E-selenium for weaning swine. Nutr. Rep. Int. 21:829.

Meydani, S. N. and A. A. Beharka. 1998. Recent developments in vitamin E and immune response. Nutr Rev 56:S49-58.

Mocchegiani, E., A. Corradi, L. Santarelli, A. Tibaldi, E. DeAngelis, P. Borghetti, A. Bonomi, N. Fabris and E. Cabassi. 1998. Zinc, thymic endocrine activity and mitogen responsiveness (PHA) in piglets exposed to maternal aflatoxicosis B1 and G1. Vet Immunol Immunopathol 62:245-260.

Molloy, C. J. and J. D. Laskin. 1985. Alterations in mouse epidermal keratin production induced by dietary vitamin A deficiency. Ann. NY Acad. Sci. 455:739.

Newsholme, E. A., P. Newsholme, R. Curi, E. Challoner, M. Salleh and M. Ardawi. 1988. A role for muscle in the immune system and its importance in surgery, trauma, sepsis and burns. Nutrition 4:261.

NRC. 1998. Nutrient Requirements of Swine.

Peplowski, M. A., D. C. Mahan, F. A. Murray, A. L. Moxon, A. H. Cantor and K. E. Ekstrom. 1980. Effect of dietary and injectable vitamin E and selenium in weanling swine antigenically challenged with sheep red blood cells. J Anim Sci 51:344-351.

Percival, S. S. 1998. Copper and immunity. Am J Clin Nutr 67:1064S-1068S.

Pleau, J. M., V. Fuentes, J. L. Morgat and J. F. Bach. 1980. Specific receptors for the serum thymic factor (FTS) in lymphoblastoid cultured cell lines. Proc Natl Acad Sci U S A 77:2861-2865.

Rojanapo, W., A. J. Lamb and J. A. Olson. 1980. The prevalence, metabolism and migration of goblet cells in rat intestine following the induction of rapid, synchronous vitamin A deficiency. J Nutr 110:178-188.

Salvin, S. B., B. L. Horecker, L. X. Pan and B. S. Rabin. 1987. The effect of dietary zinc and prothymosin alpha on cellular immune responses of RF/J mice. Clin Immunol Immunopathol 43:281-288.

Sazawal, S., R. E. Black, M. K. Bhan, S. Jalla, N. Bhandari, A. Sinha and S. Majumdar. 1996. Zinc supplementation reduces the incidence of persistent diarrhea and dysentery among low socioeconomic children in India. J Nutr 126:443-450.

Sazawal, S., R. E. Black, S. Jalla, S. Mazumdar, A. Sinha and M. K. Bhan. 1998. Zinc supplementation reduces the incidence of acute lower respiratory infections in infants and preschool children: a double-blind, controlled trial. Pediatrics 102:1-5.

Semba, R. D. 1998. The role of vitamin A and related retinoids in immune function. Nutr Rev 56: S38-48.

Shankar, A. H. and A. S. Prasad. 1998. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 68:447S-463S.

Sheffy, B. E. and R. D. Schultz. 1979. Influence of vitamin E and selenium on immune response mechanisms. Fed Proc 38:2139-2143.

Sidell, N., B. Chang and L. Bhatti. 1993. Up-regulation by retinoic acid of interleukin-2-receptor mRNA in human T lymphocytes. Cell Immunol 146:28-37.

Sijtsma, S. R., J. H. Rombout, C. E. West and A. J. van der Zijpp. 1990. Vitamin A deficiency impairs cytotoxic T lymphocyte activity in Newcastle disease virus-infected chickens. Vet Immunol Immunopathol 26:191-201.

Thies, F., L. D. Peterson, J. R. Powell, G. Nebe-von-Caron, T. L. Hurst, K. R. Matthews, E. A. Newsholme and P. C. Calder. 1999. Manipulation of the type of fat consumed by growing pigs affects plasma and mononuclear cell fatty acid compositions and lymphocyte and phagocyte functions. J Anim Sci 77:137-147.

Toepfer-Berg, T. L., J. Escobar, W. G. Van Alstine, D. H. Baker, J. L. Salak-Johnson and R. W. Johnson. submitted. Vitamin E supplementation does not mitigate the acute morbidity effects of porcine reproductive and respiratory syndrome virus in nursery pigs. J. Anim. Sci.

Tsiagbe, V. K., M. E. Cook, A. E. Harper and M. L. Sunde. 1987. Enhanced immune responses in broiler chicks fed methionine-supplemented diets. Poult Sci 66:1147-1154.

Twining, S. S., D. P. Schulte, P. M. Wilson, B. L. Fish and J. E. Moulder. 1997. Vitamin A deficiency alters rat neutrophil function. J Nutr 127:558-565.

Webel, D. M., D. C. Mahan, R. W. Johnson and D. H. Baker. 1998. Pretreatment of young pigs with vitamin E attenuates the elevation in plasma interleukin-6 and cortisol caused by a challenge dose of lipopolysaccharide. J Nutr 128:1657-1660.

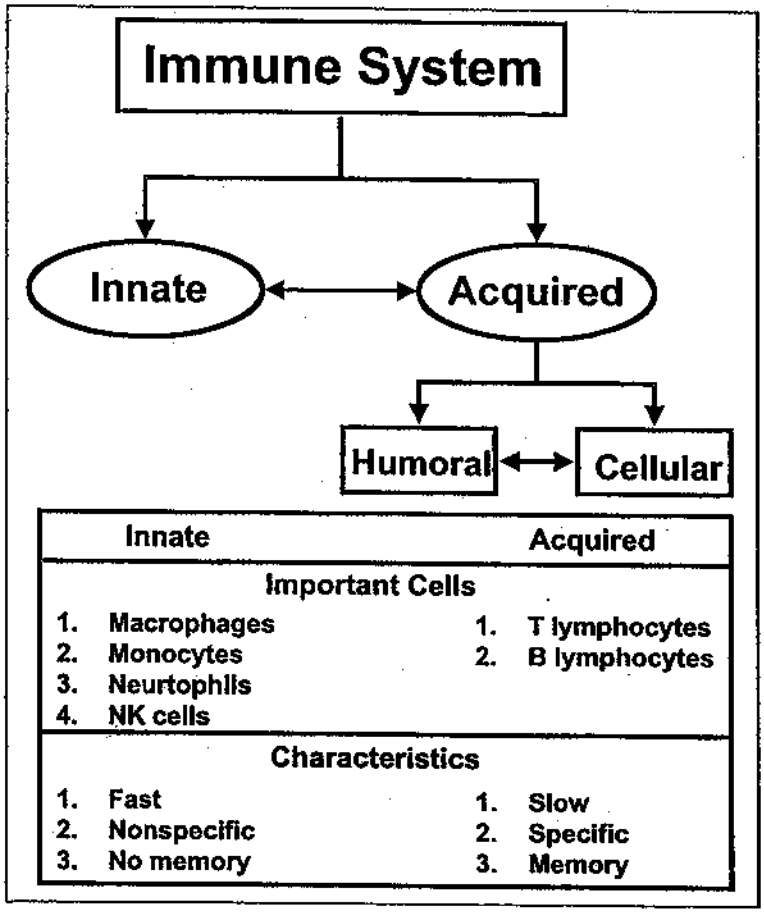
Zhao, Z., D. M. Murasko and A. C. Ross. 1994. The role of vitamin A in natural killer cell cytotoxicity, number and activation in the rat. Nat Immun 13:29-41.

Zhao, Z. and A. C. Ross. 1995. Retinoic acid repletion restores the number of leukocytes and their subsets and stimulates natural cytotoxicity in vitamin A-deficient rats. J Nutr 125:2064-2073.

Table 1. Several nutrients with well-documented immunomodulatory effects.

Nutrient	Primary immunological function
Arginine	Nitric oxide production
Glutamine	Primary nitrogen carrier in blood
n-6 PUFAs	Promote inflammation
n-3 PUFAs	Inhibit inflammation
Vitamin E & Selenium	Enhance humoral and cell-mediated immunity and inhibit inflammatory cytokine production

Figure 1. The immune system can be partitioned into two separate, but interacting components—that which provides innate immunity and that which provides acquired immunity. Both innate and acquired immunity can be modulated by nutrition.



Identifying the Limiting Amino Acids in Complex and Cereal Grain-Based Diets to Minimize Nitrogen Excretion

Gary L. Cromwell
Department of Animal Sciences
University of Kentucky
Lexington, KY 40546
859-257-7534
gcromwel@uky.edu

Summary

Addressing the potential problems of odor and environmental pollution from high levels of nitrogen (N), phosphorus (P), and other nutrients in animal manure is one of the major challenges facing animal agriculture. Swine manure is rich in N and P, and inappropriate application of manure to crop land can result in negative effects on surface and ground water. Some of the N in swine manure originates from undigested protein, but primarily it comes from the breakdown of dietary amino acids that exceeded the pig's requirements. The use of lysine and other crystalline amino acids in low protein diets is an effective method of reducing amino acid excesses, thereby lowering N excretion. However, in order to effectively use crystalline amino acids, it is important to know the order in which the amino acids are limiting when various feedstuffs and combinations of feedstuffs are fed, and the relative magnitude of difference between the amino acids in their order of limitation. This paper describes a method of obtaining this important information that must be known before one can effectively utilize supplemental amino acids in diet formulation.

Introduction

Environmental challenges in agriculture represent one of the major issues facing the animal industry. Manure and odors from swine units and other animal confinement units are two of the important environmental issues. Livestock and poultry produce nearly 160 million metric tons of manure annually in the USA (Sweeten, 1992; Table 1). Pigs excrete over 15 million tons of manure annually, which represents about 10% of the total manure production by farm animals. Large amounts of N and P are excreted in animal manure, with approximately 6.5 million tons of N and 2 million tons of P excreted annually (Table 1). Pigs are responsible for 11% (730,000 tons) of the N and 25% (460,000 tons) of the P excreted annually by farm animals.

Manure is a valuable resource when used as fertilizer to enhance crop production. Frequently, however, the nutrients that are applied to land as manure exceed the amounts needed by plants. When this happens, the excess nutrients can potentially become environmental pollutants. Excess N in soil, whether from manure, commercial fertilizer, or decomposed plant organic matter, is converted by microorganisms to ammonium ions, which are then further oxidized to nitrite and nitrate ions. Nitrates readily leach through the soil profile and can contaminate ground water. In addition, excess N in manure can result in increased emission of aerial ammonia and other gasses, including those with offensive odors (Turner et al., 1996).

Nitrogen in Swine Manure

The high N content of swine manure is attributable to several factors. First, swine diets are relatively high in protein due to the large amounts of amino acids that are needed to support a high rate of lean growth. Second, the dietary protein is not completely

digested as the feed passes through the digestive tract and endogenous N from intestinal secretions and sloughed cells are added; this results in approximately 15 to 20% of the dietary N passing out of the pig in the feces. Third, and probably most important, the pattern of the amino acids that are digested from the protein and absorbed into the bloodstream is markedly different than the pattern of amino acids needed by pigs for growth and other functions. Thus, the N from those amino acids that are in excess of the pig's requirements is converted into urea and excreted in the urine.

The amount of N that is excreted by pigs is influenced by three factors: (1) the amount of dietary N (protein) that is consumed, (2) the efficiency with which the dietary N is utilized by the animal for growth and other functions, and (3) the amount of endogenous N. In other words, the amount of excreted N can be expressed as:

N excretion = N intake - N utilized + N from endogenous sources

Generally, little can be done to influence the amount of endogenous N. Thus, in order to reduce the amount of N excreted by pigs, the amount of N that is consumed must be decreased, the efficiency of utilization of the dietary N must be increased, or both must occur.

Reducing Nitrogen Excretion

Avoid Excess Protein

There are a number of nutritional strategies that can be used to reduce the concentration of N in swine manure (CAST, 2002). One means is to feed diets that do not have excessively high levels of protein. Pigs require certain amounts of the 10 essential amino acids depending on their genetic ability to deposit lean tissue. The NRC (1998) established a system (model) for estimating the amino acids requirements of pigs based primarily on their lean growth. The daily lysine requirement at a given body weight equals the sum of the requirement for maintenance at that weight plus the requirement for whole body protein accretion, which is estimated from the carcass fat-free lean tissue deposition. The daily requirements for the other nine amino acids are based on their ratios to lysine for maintenance and for protein accretion. Daily requirements are then converted to dietary percentage requirements based on estimated daily feed intake.

Feeding pigs a high protein diet with a lysine level that exceeds the lysine requirement is of no value and may actually be a detriment. The excess amino acids are simply deaminated and the excess N is converted to urea and excreted in the urine. Every 1 percentage point increase in dietary protein beyond that needed to meet the lysine requirement results in a 10% increase in N excreted, as shown in Table 2.

Strive for Ideal Amino Acid Balance

Another means of reducing N excretion is to feed high quality protein sources having a good balance of amino acids. This will allow one to meet the lysine requirement at lower dietary protein levels than when low quality protein sources are used. For example, a corn-soybean meal diet containing 16% protein provides 0.81% lysine. However, if cottonseed meal (a poor quality protein source) is used instead of soybean meal (a high quality protein source), it takes a dietary protein level of 20% to supply the same amount of lysine (0.81%). The excesses of the other amino acids in the higher protein, cottonseed meal diet are catabolized for energy and the additional N resulting from the excess amino acids is excreted in the urine.

Some swine feeds are now being formulated on an "ideal protein" basis. An "ideal protein" is one in which the amino acids more closely match those needed by pigs for lean tissue protein synthesis and maintenance (NRC, 1998). Although nutritionists cannot formulate a diet with a perfectly balanced amino acid profile using natural feed ingredients, the use of computers and having an array of different feed ingredients allow feed manufacturers to produce feeds that have reduced amino acid excesses. Again, reduced excesses of unneeded amino acids mean that less N will be excreted.

Supplemental Amino Acids

Supplementing the diet with lysine to meet a portion of the dietary lysine requirement is one of the best strategies for reducing N excretion by pigs. This process reduces N excretion because lower protein diets can be fed when lysine is supplemented. Many research studies at the University of Kentucky and elsewhere have shown that one can reduce the protein level by 2 percentage points when the diet is supplemented with 0.15% lysine without negatively affecting performance of growing and finishing pigs (Cromwell et al., 1996; Table 3). We have also

found that one can reduce dietary protein levels by 4 percentage points without reducing performance if threonine, tryptophan, and methionine are supplemented along with lysine (Table 4). However, in these studies, carcass leanness was reduced slightly when the low protein, amino acid supplemented diets were fed. This is probably due to the fact that corn replaced 5 to 10% of the soybean meal in the lower protein diets, and corn has more net energy than soybean meal. Correcting for this difference in net energy likely would have prevented the fatter carcasses.

Feeding lower protein diets supplemented with amino acids significantly reduces N excretion. In N balance experiments conducted at the University of Kentucky (Pierce et al., 1994; Carter et al., 1996), N excretion decreased by 18 to 20% when dietary protein was reduced by 2 percentage points and lysine was added (Table 5). Furthermore, N excretion decreased by as much as 40% when the dietary protein was reduced by 4 percentage points and lysine, threonine, tryptophan, and methionine were supplemented (Table 5). According to a prediction model (Carter et al., 2003), N excretion is reduced by 10% for every 1 percentage point decrease in dietary protein, as shown in Table 2, which agrees quite well with the empirical data shown in Table 5.

Amino Acid Supplements

Lysine, as L-lysine·HCl (78% lysine), is commonly used in swine diets to supply a portion of the total dietary lysine. The price of lysine is such that it is cost effective to use in swine diets. Lysine prices, however, do fluctuate and are driven largely by the price of soybean meal. When soybean meal prices increase, there is more demand for lysine, so the price of lysine also increases.

DL-methionine or methionine hydroxy analog (MHA) is almost universally used in poultry diets because methionine is relatively inexpensive and is the first limiting amino acid in poultry diets (due to the high sulfur-amino acid content of feathers). However, methionine has less utility as a supplement for pigs because it generally is not in the upper category of limiting amino acids except in complex diets containing dried blood products (plasma or cells) or dried whey.

L-threonine and L-tryptophan have considerable potential as dietary supplements for pigs because they generally are next to lysine in their order of limitation. Until recently, the high cost of these two amino acids prohibited their usage. However, recent developments in biotechnology, new fermentation techniques, and other new technological advances, have brought these amino acids into the market place. Currently, threonine is about the same price as lysine·HCl. Tryptophan is still rather expensive (about 10 times the price of lysine and threonine), but much less is needed in diets compared with lysine and threonine.

Limiting Amino Acids – Their Order

In order to utilize crystalline amino acids in swine diets, it is important to know the order in which the amino acids are limiting when various feedstuffs and combinations of feedstuffs are fed, and the relative "distance" (or magnitude of difference) between the amino acids in that order. This information must be known before one can effectively utilize supplemental amino acids in diet formulation. For example, tryptophan is the first limiting amino acids in corn and methionine is the first limiting amino acid in soybean meal, but lysine is first limiting in a corn-soybean meal blend. For many years, nutritionists assumed that tryptophan was the second limiting amino acid in a corn-soybean meal blend, but now we know that tryptophan and threonine are almost equally limiting in their order (Russell et al., 1983, 1986). Interestingly, threonine is more limiting than tryptophan in a corn-soybean meal diet in young pigs and tryptophan is more limiting than threonine in older finishing pigs. This type of information is important for the nutritionist to know. Supplementing an amino acid that is fourth limiting is of no benefit (and may actually be a detriment) if the second and third limiting amino acids are deficient.

Table 6 shows the amino acids that are present in a corn-soybean meal diet blended to provide from 8 to 17% crude protein. By comparing the dietary amino acids at each protein level, one can approximate the order that the amino acids become limiting for a 50 kg pig. For example, going from left to right in this table (i.e., decreasing protein level and decreasing amounts of soybean meal) it is obvious that lysine is first limiting, threonine and tryptophan are next limiting, and methionine + cystine, isoleucine, and valine follow. Ample amounts of arginine and leucine and almost enough histidine and the aromatic amino acids (phenylalanine + tyrosine) are present in an all-corn diet without further protein supplementation.

Method of Determining Order of Limiting Amino Acids

Many experiments have been conducted down through the years to establish the first, second, third, etc., limiting amino acids in various feedstuffs, and the results have been published in the scientific literature. Various procedures have been used to estimate the order. Sometimes a particular feedstuff is fed with single amino acid additions and combinations of amino acid additions. In other cases, the particular feedstuff is supplemented with all questionable amino acids and single deletions of amino acids are made. Short-term growth studies, N balance studies, measurements of blood urea, and numerous other measures are often made. However, these kinds of experiments are expensive to conduct, time consuming, and require large numbers of animals. Responses are often confusing and interpretation of the data is often difficult.

Several years ago, a visual method was developed for determining the order in which amino acids become deficient and the relative magnitude of the order among amino acids (Cromwell, 1996). This rather simple method gives results that support the results of many of the empirical studies that have been conducted. It is surprisingly precise and accurate, providing that one can predict the pig's amino acids accurately and that the amino acid levels in the feedstuffs being tested are accurate.

This methodology has now been adapted to an Excel® spreadsheet, and the output is illustrated in Figures 1 and 2. The first example (Figure 1a) shows six amino acids that are provided by soybean meal in a diet containing only soybean meal at 0 to 40% of the diet (in this case, the balance of the diet would be starch or some other non-protein constituent). The percentage of the pig's requirement for each amino acid is linearly related to the level of soybean meal and is plotted for the six most limiting amino acids. The horizontal line gives the amino acid requirements for a 50 kg pig (NRC, 1998). The point at which the amino acid lines intersect the requirement line, as the percentage of soybean meal decreases from 40 to 0%, indicates the order that the amino acids in soybean meal become deficient. In other words, the order of limitation is determined by the order that the amino acids intersect the requirement line, going from right to left. In this example, the sulfur-amino acids are first limiting, threonine is second, lysine is third, and valine is fourth limiting in soybean meal protein for a 50 kg pig.

This same procedure can be used to assess the amino acids in a blend of ingredients, such as a corn-soybean meal diet. Figure 1b shows the six most limiting amino acids in this diet. For such a blend, the left vertical axis gives the percent of the requirement met by corn and the right vertical axis gives the percent of the requirement met by a soybean meal (25%) and corn (72.5%) blend. Evaluating the intersection points from right to left indicates that lysine is first limiting, threonine and tryptophan are next, and they are followed by the sulfur-amino acids, then by valine and isoleucine (equal limiting) in the corn-soybean meal blend. A wheat-soybean meal blend (Figure 1c) is first limiting in lysine and second limiting in threonine. The isoleucine, valine, and methionine + cystine requirements are nearly met and all of the other essential amino acids are more than adequate for a 50 kg pig consuming an all-wheat diet. Finally in a corn-meat meal blend (Figure 1d) tryptophan is first limiting, lysine is second, and threonine is third limiting. Isoleucine and the sulfur-amino acids are next limiting followed by valine.

Table 7 shows the six amino acids in order of the most limiting to the least limiting in commonly used cereal grains, protein supplements, and miscellaneous feed ingredients, along with various combinations of these ingredients. The requirements of a 50 kg pig are used in this table (except for the complex diets for weanling pigs), and only the six amino acids that are most likely to be limiting (lysine, threonine, tryptophan, methionine + cystine, isoleucine, and valine) are given.

The order in which the amino acids become limiting is based on the requirements for the amino acids themselves. In other words, the order changes as pigs increase in body weight and as their requirements become less. For example, as shown in Table 7 and Figure 2, the sulfur amino acids are second limiting in a corn-soybean meal diet for a 10 kg pig (although this type of diet would not likely be fed to a 10 kg pig), they are third or fourth limiting in intermediate weight pigs, and sixth limiting in a corn-soybean meal diet for a 120 kg finishing pig. The order of threonine and tryptophan also change as pigs increase in body weight, as do the order of isoleucine and valine.

This same procedure has been further refined by adapting the data to a digestible (apparent or true) amino acid basis (Figure 3). Using digestible amino acids with this procedure is useful in determining which amino acids can be supplemented and at what

levels they can be added when one is dealing with feed ingredients having certain amino acids that are poorly digestible (such as threonine in meat meal or meat and bone meal).

Economics of Amino Acid Supplementation

As indicated previously, lysine is widely used in swine feeds. The general assumption is that one can reduce the level of dietary protein in a corn-soybean meal diet by 2 percentage points and add 0.15% lysine (0.192% lysine·HCl). Another way of expressing this is that 96.15 lb of corn and 3.85 lb of lysine·HCl is equivalent to 100 lb of dehulled soybean meal in a ton of diet. Based on mid-July prices of corn ($2.40/bu; $.043/lb), soybean meal ($310/ton; $0.155/lb), and lysine·HCl ($1.20/lb), this amounts to a savings of approximately $6.75/ton.

Examples of further reductions of soybean meal in diets with additional amino acids are shown Table 8. This table shows the amounts of lysine, threonine, tryptophan, and methionine that can replace soybean meal in corn-soybean meal diets for 20, 50, 80, and 120 kg pigs. These amounts of amino acids are based on the soybean meal that can be eliminated from the diet (and replaced with corn) while still meeting the requirement for the fifth limiting amino acid, either isoleucine or valine.

Based on the mean of the four weight groups, soybean meal can be reduced by approximately 10.5% (equivalent to a 4% reduction in dietary protein). With this reduction, 0.38% lysine·HCl, 0.10% threonine, 0.035% tryptophan, and 0.02% methionine would need to be added. Expressed another way, 199.3 lb of corn, 7.6 lb of lysine·HCl, 2.0 lb of threonine, 0.7 lb of tryptophan, and 0.4 lb of methionine could replace 210 lb of soybean meal in a ton of diet. At mid-July prices of corn, soybean meal, and amino acids (threonine = $1.40/lb, tryptophan = $13.00/lb, methionine = $1.10/lb), the substitution of corn and these four amino acids for soybean meal is slightly more economical ($30.03 vs $32.50, respectively, for 210 lb of substitution). However, at more typical prices of corn ($2.00/bu) and soybean meal ($200/ton), amino acid supplementation is more costly than the intact protein diet ($28.58 vs $21.00 for 210 lb substitution).

In summary, knowing the limiting amino acids in cereal grains, protein supplements, by-product feeds and combinations of feed ingredients, and understanding the order that amino acids become limiting as the dietary protein level is reduced is essential in order to utilize supplemental amino acids effectively in low protein diets that minimize N excretion.

References

Carter, S.D., G.L. Cromwell, M.D. Lindemann, L.W. Turner, and T.C. Bridges. 1996. Reducing N and P excretion by dietary manipulation in growing and finishing pigs. J. Anim. Sci. 74 (Suppl. 1):59 (abstr.).

Carter, S., P. Westerman, T. van Kempen, G. Shurson, G. Cromwell, G. Hill, K. Casey, and B. Richert. Fass-ASAE Manure Standards Review Committee. 2002. Estimation of manure nutrient excretion from swine based upon diet composition and feed intake. J. Anim. Sci. 80 (Suppl. 1):550 (abstr.).

Carter, S.D, G.L. Cromwell, P.W. Westerman, J.S. Park, and L.A. Pettey. 2003. Prediction of nitrogen, phosphorus, and dry matter excretion by swine based on diet chemical composition, feed intake, and nutrient retention. Proc. 9th International Symposium on Animal, Agricultural, and Food Processing Wastes, American Society of Agricultural Engineers, Raleigh, NC. pp 285–295.

CAST. 2002. Animal diet modification to decrease the potential for nitrogen and phosphorus pollution. Issue Paper No. 21. Council for Agricultural Science and Technology, Ames, IA.

Cromwell, G.L., M.D. Lindemann, G.R. Parker, K.M. Laurent, R.D. Coffey, H.J. Monegue, and J.R. Randolph. 1996. Low protein, amino acid supplemented diets for growing-finishing pigs. J. Anim. Sci. 74 (Suppl. 1):174 (abstr.).

Cromwell, Gary L. 1996. Low protein, amino acid supplemented diets for growing-finishing pigs. Proc. 16th Prince Agri Feed Ingredient Conference, Quincy, IL. pp. 13-26.

NRC. 1998. Nutrient Requirements of Swine. 10th ed. National Academy Press, Washington, DC.

Pierce, J.L., K.L. Enright, G.L. Cromwell, L.W. Turner, and T.C. Bridges. 1994. Dietary manipulation to reduce the N and P excretion by finishing pigs. J. Anim. Sci. 72 (Suppl. 1):331 (abstr.).

Russell, L.E., G.L. Cromwell and T.S. Stahly. 1983. Tryptophan, threonine, isoleucine and methionine supplementation of a 12% protein, lysine-supplemented, corn-soybean meal diet for growing pigs. J. Anim. Sci. 56:1115-1123.

Russell, L.E., R.A. Easter, V. Gomez-Rojas, G.L. Cromwell and T.S. Stahly. 1986. A note on the supplementation of low-protein, maize-soyabean meal diets with lysine, tryptophan, threonine and methionine for growing pigs. Anim. Prod. 42:291-295.

Sweeten, J.M. 1992. Livestock and Poultry Waste Management: A National Overview. In: J. Blake, J. Donald, and W. Magette (Ed.) National Livestock, Poultry and Aquaculture Waste Management pp 4-15. Amer. Soc. Agric. Eng., St. Joseph, MI.

Turner, L.W., G. L. Cromwell, T. C. Bridges, S. Carter, and R. S. Gates. 1996. Ammonia (NH_3) emission from swine waste as influenced by diet manipulation. Proc. 1st International Conference on Air Pollution from Agricultural Operations. Kansas City, MO. Feb. 5-9, 1996. MidWest Plan Service, Iowa State Univ., Ames. pp. 453-458.

Table 1. Quantities of manure, nitrogen, and phosphorus excreted annually by livestock and poultry in the United States (dry matter basis)[a]

Species	Manure (million tons)	Concentration (%) N	Concentration (%) P	Excretion (thousand tons) N	Excretion (thousand tons) P
Ruminants					
Beef cattle	96.6	3.96	1.07	3,828	1,029
Dairy cattle	29.1	3.75	0.79	1,091	230
Sheep	1.8	3.89	0.56	70	10
Nonruminants					
Swine	15.5	4.71	2.97	730	460
Poultry	15.4	5.13	1.62	790	250
Total	158.4			6,509	1,979

[a]Adapted from Sweeten (1992).

Table 2. Nitrogen excretion of pigs from 20 to 120 kg[a]

Dietary protein level fed	N excreted, kg	Change
Correct percentage of protein to meet the lysine requirement	4.62	
1% more protein than needed	5.08	10% more N
2% more protein than needed	5.54	20% more N
2% less protein + lysine	3.70	20% less N
4% less protein + lysine, threonine, tryptophan, and methionine	2.78	40% less N

[a]Carter et al. (2002, 2003). Based on NRC (1998) standards and a N/P excretion model developed by S.D. Carter (Oklahoma State University) and G.L. Cromwell (University of Kentucky). Predicted N excreted is N consumed minus whole empty body N retained by barrows and gilts averaging 50% fat-free carcass lean and 325 grams of fat-free carcass lean gain per day.

Table 3. Performance of growing-finishing pigs fed low protein, corn-soybean meal diets without and with supplemental lysine[a]

Item	Dietary protein, %		
	16-14	14-12	14-12 + 0.15% lysine
Daily gain, kg	0.74	0.67	0.74
Daily feed, kg	2.27	2.32	2.28
Feed/gain	3.09	3.47	3.10
Carcass (scanned)			
Backfat, cm	2.64	-	2.79
Loin depth, cm	4.78	-	4.85
Estimated lean, %	48.2	-	47.4

[a]University of Kentucky. Four experiments, 78 pigs per treatment, 19 to 103 kg body weight. Protein levels were reduced to from 16 to14, from 14 to 12, and from 14 to 12%, respectively, at 50 kg. Carcass data from one experiment.

Table 4. Performance and carcass traits of pigs fed low protein, corn-soybean meal diets supplemented with amino acids[a]

Item	Dietary protein	
	Adequate[b]	Low[c] + amino acids[d]
Daily gain, kg	0.74	0.75
Daily feed, kg	2.27	2.29
Feed/gain	3.06	3.07
Carcass (scanned)		
Backfat, cm	2.51	2.77
Loin depth, cm	4.67	4.75
Estimated lean, %	48.9	47.4

[a]Seven experiments at the University of Kentucky involving growing pigs (17 to 34 kg), finishing pigs (53 tp 94 kg), and growing-finishing pigs (21 to 109 kg); 184 pigs per treatment. Carcass data based on three trials with finishing pigs.
[b]Adequate = 16 to17% protein in grower, 14 to 15% in developer, and 13 to 14% in finisher.
[c]Low = 4 percentage points less protein than adequate diet.
[d]Added lysine (0.30%), threonine (0.05 to 0.10%), tryptophan (0.03 to 0.05), and methionine (0.05 to 0.10%).

Table 5. Nitrogen excretion of pigs fed reduced protein diets with amino acids

	N excretion			
	Fecal N g/day	Urinary N g/day	Total N g/day	Reduction %
Experiment 1[a]				
71 kg pigs				
14% protein	4.7	16.2	20.9	
12% protein + lys	4.0	12.8	16.8	20
10% protein + lys, thr, and trp	4.6	9.4	14.0	33
108 kg pigs				
14% protein	4.3	19.1	23.4	
12% protein + lys	4.5	14.6	19.1	18
10% protein + lys, thr, and trp	5.2	12.3	17.5	25
Experiment 2[b]				
34 kg pigs				
16% protein	5.7	11.2	16.9	
12% protein + lys, thr, trp, and met	5.4	5.7	11.1	34
115 kg pigs				
14% protein	7.3	22.1	29.4	
10% protein + lys, thr, trp, and met	5.6	11.9	17.5	40

[a]Pierce et al. (1994). The 12% protein diet was supplemented with 0.15% lysine and the 10% protein diet was supplemented with 0.30% lysine, 0.08% threonine, and 0.03% tryptophan.
[b]Carter et al. (1996). The low protein diets were supplemented with 0.30% lysine, 0.10% threonine, 0.05% tryptophan, and 0.10% methionine.

Table 6. Essential amino acids in corn-soybean meal diets containing various protein levels[abc]

Amino acid	Requirement 50 kg pig	Dietary protein, %									
		17	16	15	14	13	12	11	10	9	8
Lysine	**0.84**	0.88	0.81	0.74	0.67	0.60	0.53	0.46	0.39	0.32	0.25
Arginine	**0.32**	1.07	0.99	0.91	0.83	0.75	0.67	0.59	0.51	0.43	0.37
Histidine	**0.27**	0.46	0.44	0.41	0.38	0.36	0.33	0.30	0.28	0.25	0.22
Isoleucine	**0.46**	0.70	0.65	0.60	0.56	0.51	0.46	0.41	0.37	0.32	0.27
Leucine	**0.80**	1.57	1.50	1.44	1.37	1.30	1.23	1.16	1.10	1.03	0.97
Met + Cys	**0.48**	0.59	0.56	0.54	0.51	0.48	0.46	0.43	0.40	0.38	0.35
Phe + Tyr	**0.77**	1.44	1.35	1.25	1.16	1.07	0.98	0.89	0.80	0.71	0.62
Threonine	**0.55**	0.64	0.60	0.56	0.52	0.48	0.44	0.40	0.36	0.32	0.28
Tryptophan	**0.15**	0.19	0.18	0.16	0.15	0.13	0.12	0.10	0.09	0.07	0.06
Valine	**0.57**	0.81	0.76	0.71	0.66	0.62	0.57	0.52	0.47	0.42	0.38

[a]Amino acid (total) requirements of a 50 kg pig of high-medium lean growth rate (325 g/day of carcass fat-free lean) and consuming a fortified corn-soybean meal diet containing 2.5% minerals, vitamins, and additives (3,400 kcal DE/kg) (NRC, 1998).
[b]Amino acids in shaded areas represent deficient levels.
[c]The 17% protein diet consists of 74.8% corn and 22.8% dehulled soybean meal and the 8% protein diet consists of 97.5% corn and 0.0% dehulled soybean meal. Every 1% increase in soybean meal represents an increase of 0.39% dietary protein. Similarly, every 1% change in dietary protein represents a change of 2.53% in soybean meal and a change of 0.07% in lysine.

Figure 1a – Soybean meal

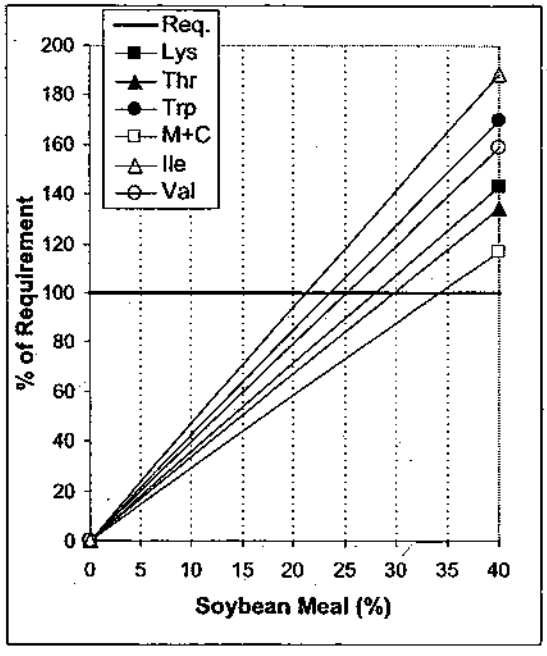


Figure 1b – Corn-soybean meal diet

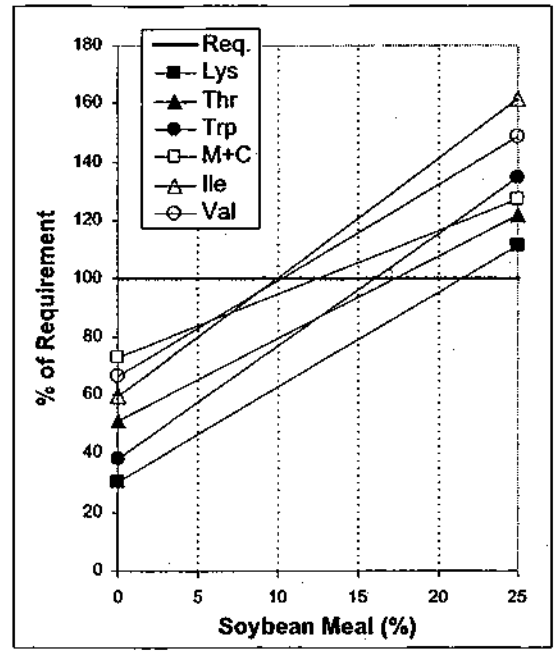


Figure 1c – Wheat-soybean meal diet

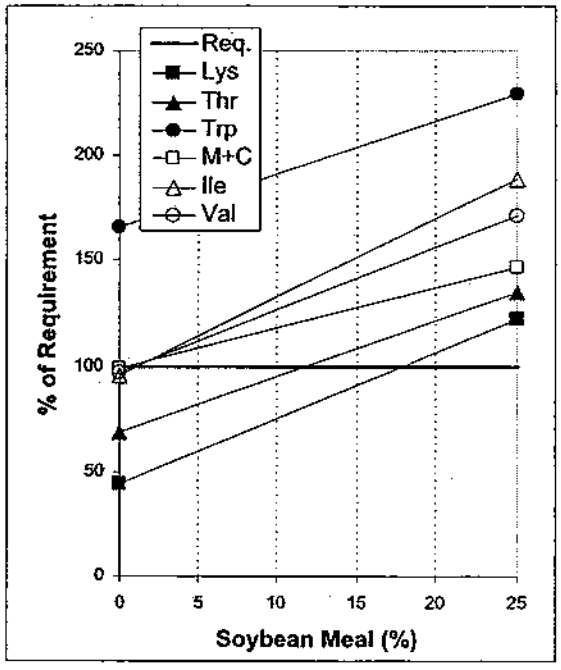


Figure 1d – Corn-meat meal diet

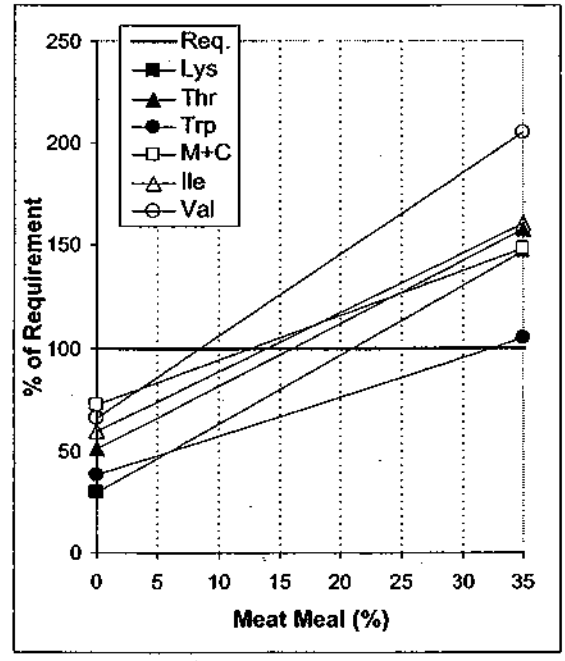


Figure 1. Procedure for determining the limiting amino acids as the level of soybean meal or meat meal is reduced in the diet for a 50 kg pig. The order in which amino acids become limiting is indicated by the point at which the amino acid crosses the horizontal line (100% of the requirement) proceeding from right to left.

Figure 2a – Corn-soy diet – 10 kg pig

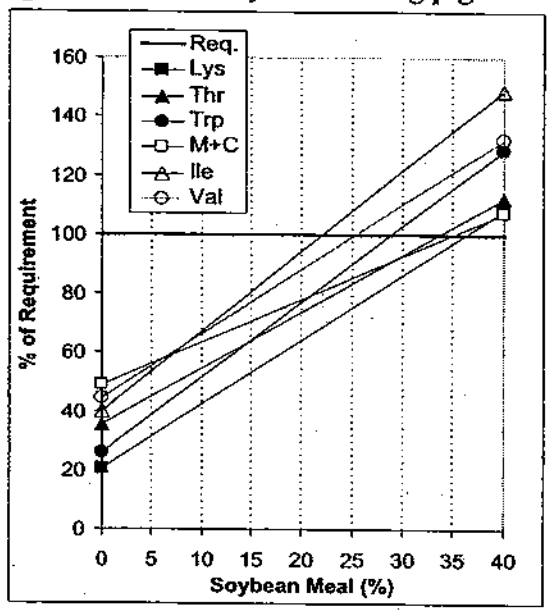


Figure 2b – Corn-soy diet – 20 kg pig

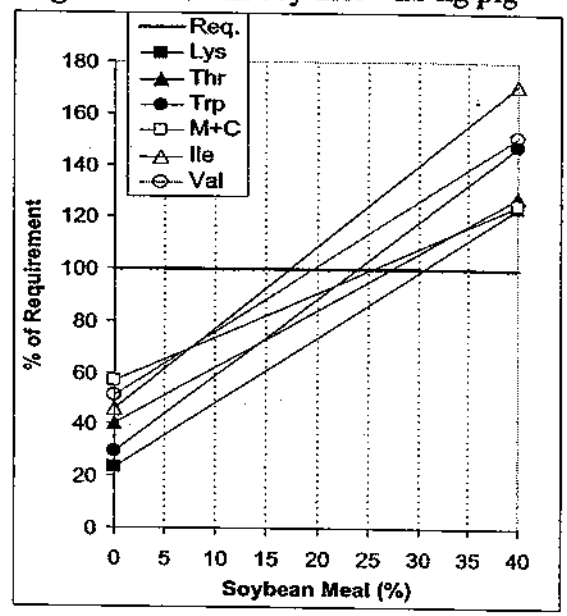


Figure 2c – Corn-soy diet – 50 kg pig

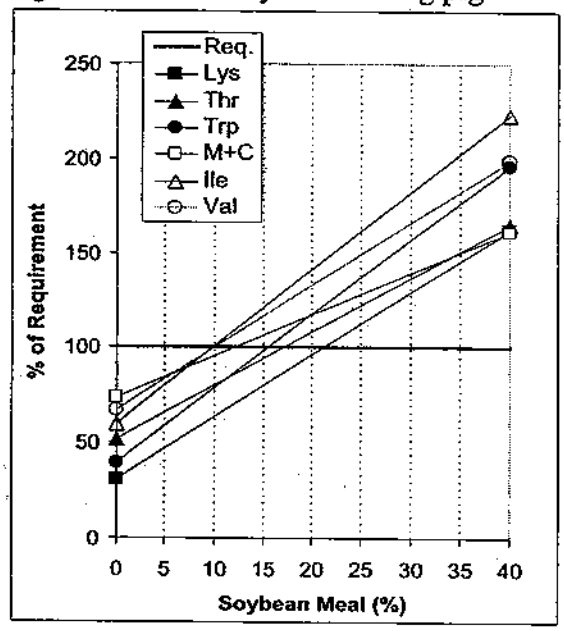


Figure 2d – Corn-soy diet – 110 kg pig

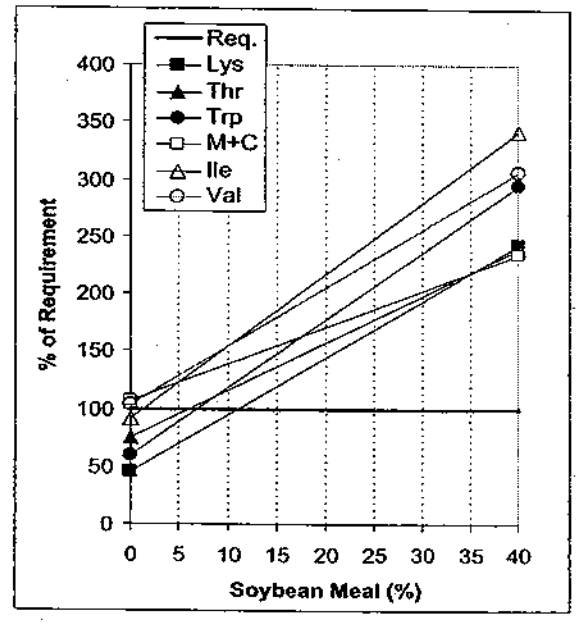


Figure 2. Limiting amino acids as the level of soybean meal is reduced in a corn-soybean meal diet for a 10, 20, 50, and 110 kg pig. The order in which amino acids become limiting is indicated by the point at which the amino acid crosses the horizontal line (100% of the requirement) proceeding from right to left.

Figure 3a – Total amino acids (AA) basis

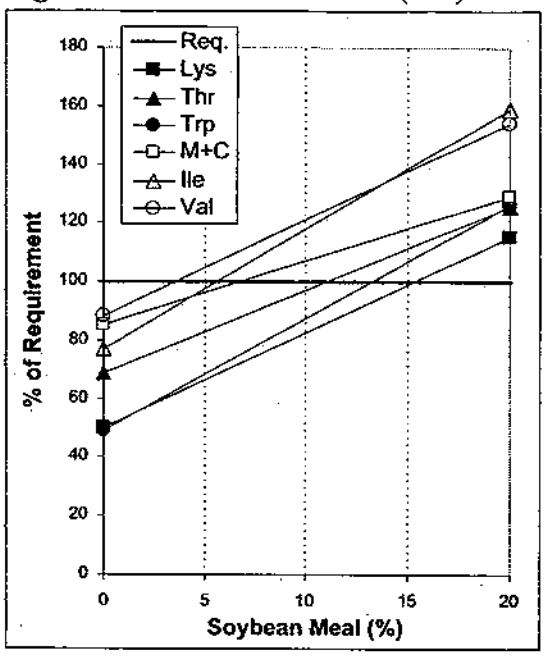


Figure 3b – Apparent digestible AA basis

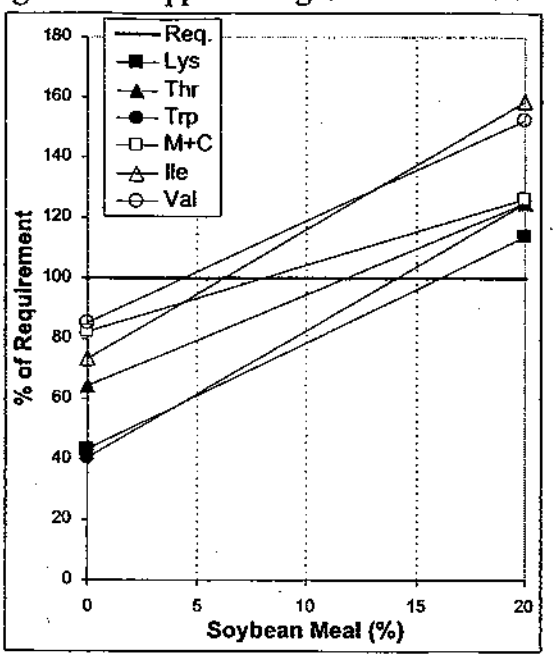


Figure 3c – True digestible AA basis

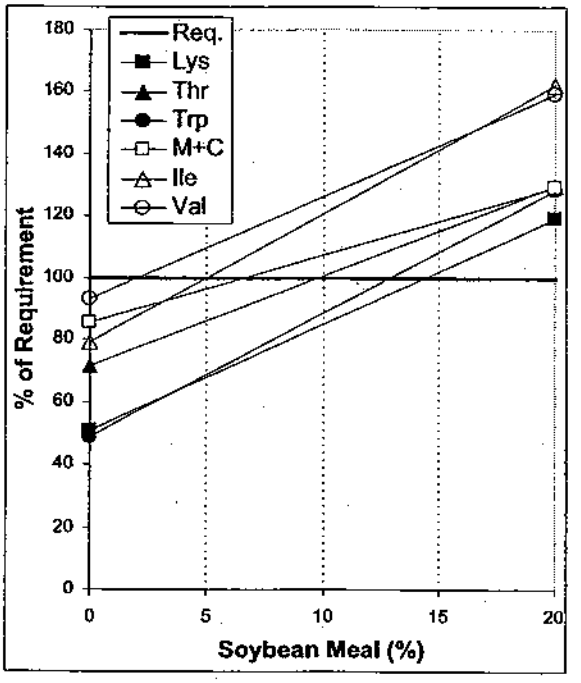


Figure 3. Limiting amino acids as the level of soybean meal is reduced in a corn-soybean meal diet containing 7.5% meat and bone meal. The three figures illustrate differences in the magnitude of the order of limiting amino acids based on total (Figure 4a), apparent ileal digestible (Figure 4b), and true ileal digestible (Figure 4c) amino acids for a 50 kg pig. The order in which amino acids become limiting is indicated by the point at which the amino acid crosses the horizontal line (100% of the requirement) proceeding from right to left.

Table 7. Limiting amino acids in selected feed ingredients, simple diets, and complex diets for swine[ab]

	Limiting amino acids					
	First	Second	Third	Fourth	Fifth	Sixth
Cereal grains						
Corn	Lys	Trp	Thr	Ile	Val	M+C
Sorghum	Lys	Thr	Trp	M+C	(Val	Ile)
Wheat	Lys	Thr	(Ile	Val	M+C)	Trp
Barley	Lys	Thr	M+C	Ile	(Trp	Val)
Oats	Lys	Thr	Trp	Ile	Val	M+C
Protein sources						
Soybean meal	M+C	Thr	Lys	Val	Trp	Ile
Canola meal	Lys	(Thr	Trp)	(Ile	Val)	M+C
Cottonseed meal	Lys	Thr	(Ile	M+C)	(Val	Trp)
Meat meal	Trp	M+C	(Ile	Thr	Lys)	Val
Meat and bone meal	Trp	M+C	(Thr	Ile	Lys)	Val
Blood meal	Ile	M+C	Thr	Lys	Trp	Val
Fish meal	Trp	(Thr	M+C)	Val	(Ile	Lys)
Miscellaneous						
Dried plasma	Ile	M+C	Lys	(Thr	Val)	Trp
Dried blood cells	Ile	M+C	Thr	Trp	Lys	Val
Dried whey	M+C	(Lys	Val)	Trp	Thr	Ile
Simple diets						
Corn-soybean meal	Lys	Thr	Trp	M+C	(Val	Ile)
Corn-canola	Lys	Trp	Thr	Ile	Val	M+C
Corn-meat meal	Trp	Lys	Thr	Ile	M+C	Val
Corn-meat and bone meal	Trp	Lys	Thr	Ile	M+C	Val
Corn-fish meal	Trp	Lys	Thr	Ile	Val	M+C
Corn-cottonseed meal	Lys	Thr	Trp	Ile	(Val	M+C)
Sorghum-soybean meal	Lys	Thr	M+C	Trp	Val	Ile
Wheat-soybean meal	Lys	Thr	(Ile	Val	M+C)	Trp[c]
Barley-soybean meal	Lys	Thr	M+C	(Ile	Val	Trp)
Oats-soybean meal	Lys	Thr	Trp	Ile[c]	Val[c]	M+C[c]
Corn-soybean meal + 5% fish meal	Lys	Trp	Thr	M+C	(Ile	Val)
Corn-soybean meal + 5% meat meal	Lys	Trp	Thr	M+C	Ile	Val
Complex diets						
Corn-soy + 30% dried whey[d]	M+C	Lys	Thr	(Trp	Val)	Ile
Corn-soy + 25% whey + 6% plasma[d]	M+C	Thr	(Trp	Val)	Lys	Ile
Corn-soy + 10% whey + 3% cells[e]	M+C	Thr	Trp	Lys	Val	Ile

Table 7 (continued)

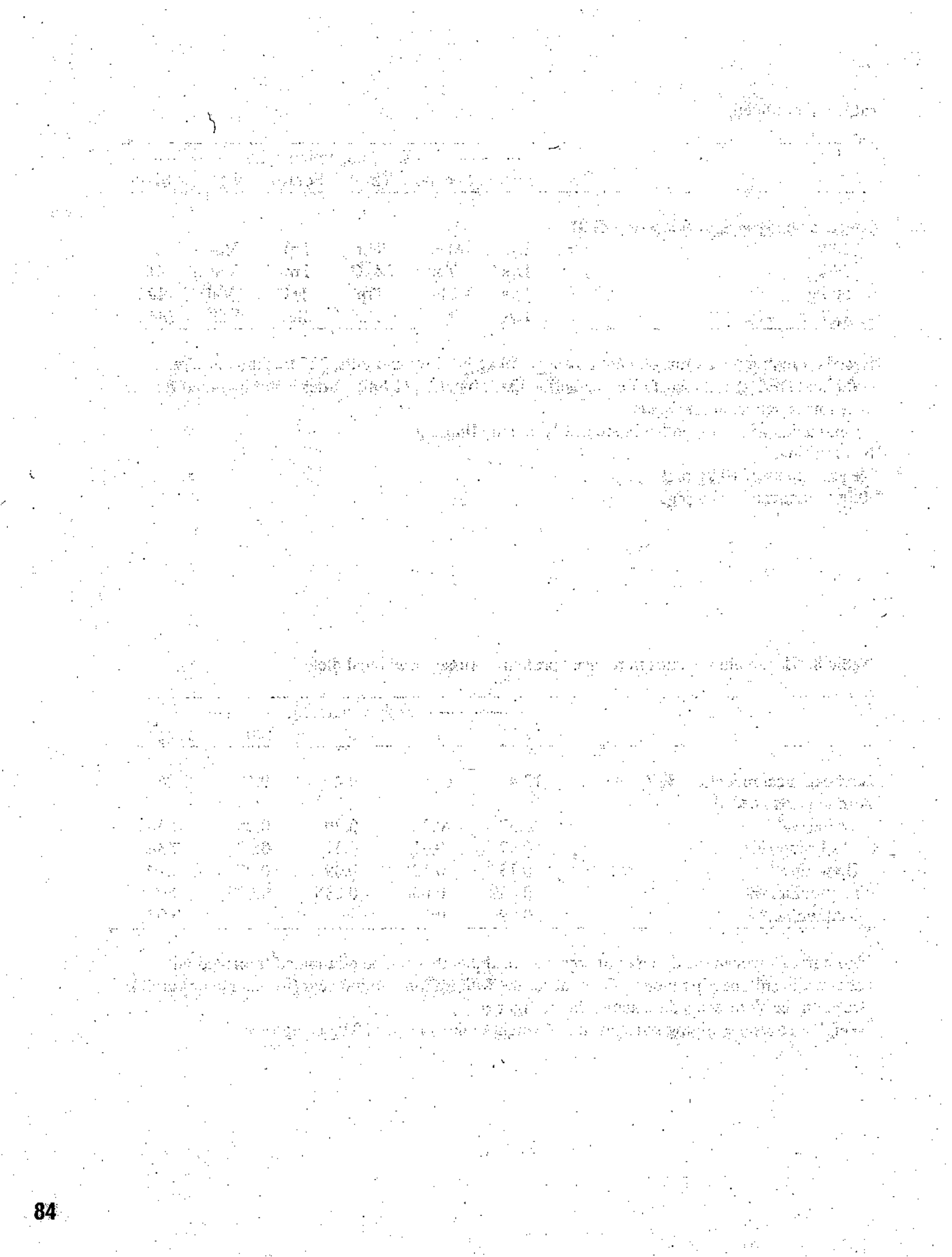
	Limiting amino acids					
	First	Second	Third	Fourth	Fifth	Sixth
Effects of body weight (corn-soy diet)						
10 kg	Lys	M+C	Thr	Trp	Val	Ile
20 kg	Lys	Thr	M+C	Trp	Val	Ile
50 kg	Lys	Thr	Trp	M+C	(Val	Ile)
120 kg	Lys	Trp	Thr	Ile	Val[c]	M+C[c]

[a]Based on requirements for total amino acids (50 kg barrows and gilts, 325 g lean gain/day, 3,400 kcal DE/kg) and feedstuff composition listed by NRC (1998). Order is not included for the other four essential amino acids.
[b]Amino acids within parentheses are nearly equally limiting.
[c]Not limiting.
[d]Requirements of 10 kg pigs.
[e]Requirements of 20 kg pigs.

Table 8. Use of amino acids in reduced protein, corn-soybean meal diets

	Body weight, kg				
	20	50	80	120	Avg[b]
Soybean meal reduction, %[a]	13.4	11.5	10.4	9.1	10.6
Amino acids needed					
Lysine, %[b]	0.37	0.32	0.29	0.25	0.30
As lysine·HCl	0.47	0.41	0.37	0.32	0.38
Threonine, %	0.15	0.12	0.09	0.07	0.10
Tryptophan, %	0.039	0.036	0.035	0.033	0.035
Methionine, %	0.09	0.03	-	-	0.02

[a]Amount of soybean meal in a corn-soybean meal diet that can be eliminated (replaced with corn) while still meeting the requirement for the fifth limiting amino acid (isoleucine or valine). Based on the slope procedure shown in the figures.
[b]Weighted average, giving twice as much weight to the 80 and 120 kg categories.



Phytase and Phosphorus Movement in Soil

Brad C. Joern[1] and Alan L. Sutton[2]
[1]Department of Agronomy
Purdue University
West Lafayette, IN 47907
765-494-9767
bjoern@purdue.edu
[2]Department of Animal Sciences

Summary

Phosphorus (P) loss from soils can adversely affect the trophic status and potential use(s) of freshwater ecosystems. If applied at excessive P rates to cropland, manure can significantly increase soil P loss potential. Considerable research has demonstrated that P loss potential is related to the time, rate and method of manure applied, as well as field characteristics like soil test P, runoff and erosion potential, distance to waterbody, and the presence of tile drains or other direct hydrological connections. Phytase has been used to improve dietary P availability and reduce total manure P excretion in swine and poultry. However, phytase also can impact the form(s) of P excreted in manure, and therefore, may impact potential P loss from soils. In this paper, we provide an overview of the environmental consequences of excess P, pathways of P transport to surface and ground water resources, the role of phytase in reducing manure P excretion by pigs, and the potential impact of phytase on P movement in manure-amended soils.

Phosphorus and the Environment

Phosphorus (P) is an essential element for normal growth, development, and reproduction of both plants and animals. However, excessive P levels can impair surface water quality. It is well established that P is the limiting nutrient for phytoplankton production in lakes (Vollenweider, 1968; Schindler, 1974, 1977). Although less data exist for streams and rivers, research indicates P also is a limiting nutrient in these systems (Stockner and Shortreed, 1978; Elmwood et al., 1981; Peterson et al., 1985). High P levels in surface waters accelerate the eutrophication process and often result in excessive growth of phytoplankton like algae and cyanobacteria. The respiration of these organisms leads to decreased oxygen levels in bottom waters, and under certain circumstances (at night under calm, warm conditions) in surface waters (Correll, 1998). These decreased oxygen levels can lead to fish kills and significantly reduce aquatic organism diversity.

Both organic and inorganic P compounds are present in soil; however, plants take up only inorganic P. Soil P dynamics are largely influenced by soil pH, clay content and mineralogy, amorphous iron, aluminum and manganese, organic matter, and soil moisture. Inorganic P is the predominant P form in both manures and commercial fertilizers. Depending on soil pH and mineralogy, inorganic P can be sorbed to the surface of clays and amorphous iron and aluminum compounds, or be precipitated as mineral salts until they are utilized by plants. Organic forms of P from crop residues, soil organic matter and manures can be mineralized to inorganic P by soil microorganisms and become available for plant uptake. Conversely, inorganic P can be immobilized to organic P forms that are not available for plant uptake. In addition, some organic P forms excreted in manure may displace previously sorbed inorganic P from soils and increase inorganic P runoff and/or leaching in the soil, while other organic P forms may not be readily sorbed by the soil and move more rapidly via runoff or leaching.

Much of the P reaching water is from runoff, often with sediment, from cropland receiving high rates of manure or inorganic fertilizers. The extent of P runoff from soils depends on rainfall intensity, soil type, topography, soil moisture content, crop cover, and the form, rate and method of P application. Con-

servation best management practices that reduce surface runoff and erosion can greatly reduce the risk of P loss from soils. While P loss to surface and ground water via P leaching through the soil profile is generally much smaller than runoff P losses, excessive P applications to soils over time will move P to lower portions of the soil profile, and this P can discharge into tile drains, ditches and eventually streams. Significant P discharges from tile drains also can occur via macropore transport of manure to tile lines after land application, especially during the dry season when cracks form in the topsoil. Additionally, sandy soils with rapid drainage generally have greater P leaching potential than heavier textured soils.

Phosphorus and US Swine Production

Approximately 60 million pigs are present on US farms at any one time and they generate approximately 85 million tonnes of manure, including 700,000 tonnes of N, 450,000 tonnes of P_2O_5 and 370,000 tonnes of K_2O each year (Hess et al., 2001). Unfortunately swine manure N, P_2O_5 and K_2O composition is not properly balanced for plant uptake by typical crops grown in production agriculture. The relative ratio of potentially available N, P_2O_5 and K_2O in manure from pigs fed commercial diets after storage in liquid pits is roughly 1:1:1. Additional N losses resulting from alternative manure storage systems, as well as manure application method and timing can result in relative N, P_2O_5 and K_2O ratios closer to 1:2:2. Corn grain production requires roughly a 3:1:1 ratio of N, P_2O_5 and K_2O, and if corn is grown for silage, then a 2:1:2 ratio of N, P_2O_5 and K_2O is desirable. Therefore, when manure is applied to meet the N requirements of crops, the P applied nearly always exceeds crop removal. For example, if manure is applied to meet the N requirement for corn grain, manure P applications may be 3-6 times greater than crop P removal.

In certain regions of the US, more P is produced in manure than can be utilized by productive cropland; however, most manure P loading challenges occur at the farm or community level. In the past 15-20 years, pork producers have increased the number of pigs at individual farms more rapidly than they have increased the land area available for manure application. As a result, manure applications have been made more frequently to individual fields.

Frequent applications of manure to cropland, especially at N based rates, rapidly increase soil test P levels and increase potential P loss from agricultural fields. Increased soil P accumulation at the farm, community and regional levels has prompted efforts to regulate or encourage the long-term management of manure applications on a P basis through the recent revision of the USEPA CAFO NPDES permit rule and the promotion of NRCS sponsored comprehensive nutrient management plans (CNMPs) to improve nutrient balance and management at the farm level.

Phytase Impacts on Manure Phosphorus

In response to both regulatory and voluntary management efforts to reduce P loading to agricultural soils, phytase has become more commonplace in monogastric (swine and poultry) diets. Compared to industry standard diets, phytase inclusion, with a concomitant reduction in inorganic P supplementation, can reduce manure P excretion 20-30 percent without affecting animal performance. Phytase also can change the form(s) of P excreted in manure, and some concern does exist about whether these changes in manure P composition may influence P movement in manure-amended soils.

While some studies have shown that phytase additions have the potential to increase water soluble P (WSP) and P runoff losses in poultry litter (Moore et al., 1998; Delaune et al., 2001), other research has shown that WSP levels in poultry litter are more closely associated with the dietary P level fed rather than the use of phytase (Applegate et al., 2003). In studies involving pigs, most research has shown that with appropriate reductions in supplemental inorganic P, phytase inclusion either decreases, or has no effect on, WSP excretion (Baxter et al., 1998; Hankins, 2001; Hill et al., 2003; Nussbaum-Wagler, 2003).

Phytase Impacts on Phosphorus Loss From Swine Manure-Amended Soils

While several studies have been conducted to determine the impact of phytase on P runoff from pastures that receive poultry litter, only a few studies have evaluated the impact of phytase on P loss from tilled soils. Gilley et al. (2001) measured the amount of P and N lost from soils that received swine slurry from animals fed a traditional diet or a diet containing phytase when the slurries were applied on an equal P basis to soils. Although more N was lost from the soils receiving the phytase diet, they did not

observe significant differences in the amount of P lost from soils receiving either diet. Nussbaum-Wagler (2003) measured the amount of N, P and sediment lost from soils that received swine slurry from pigs fed a standard or improved available P diet that included phytase. Manures were surface broadcast or incorporated into the soil and applied on an equal N basis. Soils amended with manure from the standard diet had WSP and total P losses that were roughly 1.5 and 1.3 times greater than soils amended with manure from the improved available diet. These reductions in P loss were largely attributed to the reduced amount of P applied in the manure from the improved diet.

From these manure application studies conducted on tilled soils that are planted to annual crops, P losses appear to be more closely related to the total quantity of P applied in manure, rather than differences in the form of P applied in manure. In the Nussbaum-Wagler (2003) study, manure placement (surface broadcast vs. incorporated) had a far greater impact on P loss than diet.

Our Practical Knowledge About Phytase and Soil Phosphorus Movement

Phytase can significantly reduce manure total P excretion, and therefore, manure P loading to soils. The overall reduction in soil P loading from phytase use will positively impact the environment. Most research also shows that phytase reduces P loss potential when manure application rates are N-based, as total and soluble manure P additions are both generally lower from phytase diets under this scenario. However, when manure application rates are P-based, P loss potential may be greater when manure from a phytase diet is applied compared to a standard diet. The primary reasons for this increased risk of P loss are 1) our lack of knowledge about the true non-phytate P replacement value of phytase in various diets, and 2) our lack of willingness to feed at the true available P needs of the animal. When diets including phytase are developed with a relatively large safety margin, greater amounts of manure WSP can be generated, and this P can be readily lost to surface waters, especially when manure is applied on the soil surface just prior to a runoff inducing precipitation event. However, since most states encourage or allow manure to be applied on a N rate basis, with P rate limits managed by the frequency of manure application, the risk of increased P loss as a result of phytase use likely will be limited.

References:

Applegate, T.J., B.C. Joern, D.L. Nussbaum-Wagler, and R. Angel. 2003. Water-soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. Poultry Sci. 82:1024-1029.

Baxter, C.A., B.C. Joern, and O. Adeola. 1998. Dietary phosphorus management to reduce soil phosphorus loading from pig manure. pp. 104-110. *In* D. Franzen (ed.) Proceedings of the Twenty-Seventh North Central Extension-Industry Soil Fertility Conference.

Correll, D.L. 1998. The role of phosphorus in the eutrophication of receiving waters: A review. J. Environ. Qual. 27:261-266.

DeLaune, P.B., P.A. Moore, Jr., D.C. Carman, T.C. Daniel, and A.N. Sharpley. 2001. Development and validation of a phosphorus index for pastures fertilized with animal manure. Proc. Intl. Symp. Addressing Anim. Prod. Environ. Issues.

Elmwood, J.W., J.D. Newbold, A.F. Trimble, and R.W. Stark. 1981. The limiting role of phosphorus in a woodland stream ecosystem: Effects of P enrichment on leaf decomposition and primary producers. Ecology. 62:146-158.

Gilley, J.E., B. Eghball, B.J. Wienhold, and P.S. Miller. 2001. Nutrients in runoff following the application of swine manure to interrill areas. Trans. of the ASAE. 44:1651-1659.

Hankins, S. L. 2001. Dietary Manipulation Effects on Nutrient Excretion and Performance of Beef Cattle and Pigs. MS thesis, Purdue University, West Lafayette, IN.

Hess, P.J., B.C. Joern, and J.A. Lory. 2001. Developing software for livestock manure and nutrient management in the USA. pp. 371-389. *In* R.F. Follet and J.L. Hatfield (ed.) Nitrogen in the Environment: Sources, Problems, and Management. Elsevier Science B.V., Amsterdam, The Netherlands.

Hill, B. E., S. L. Hankins, S. A. Trapp, A. L. Sutton and B. T. Richert. 2003. Effects of low phytic acid corn, low phytic acid soybean meal, and phytase on nutrient digestibility and excretion in growing pigs. J. Anim. Sci. 81 (Suppl. 1):37. (Abstract).

Moore, P. A., W. E. Huff, W. F. Jaynes, G. R. Huff, J. M. Balog, N. C. Rath, T. C. Daniel, P. W. Waldroup, L. S. Davis and D. R. Edwards. 1998. Effect of high available phosphorus corn and phytase enzyme addition to broiler diets on phosphorus runoff from fescue plots. Poult. Sci. S30.

Nussbaum-Wagler, D. 2003. Phosphorus Runoff from Tilled Soils under Simulated Rainfall. MS Thesis. Purdue University, West Lafayette, IN.

Peterson, B.J., A.E. Hobbie, A.E. Hershey, M.A. Lock, T.E. Ford,, J.R. Vestal, V.L. McKinly, M.A.J. Hullar, M.C. Miller, R.M. Ventullo, and G.S. Volk. 1985. Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. Science. (Washington D.C.) 229:1383-1386.

Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science 195:260-262.

Schindler, D.W. 1974. Eutrophication and recovery in experimental lakes: Implications for lake management. Science (Washington D.C.) 184:897-899.

Stockner, J.G. and K.R.S. Shortreed. 1978. Enhancement of autotrophic production by nutrient addition in a coastal rainforest stream on Vancouver Island. J. Fish Res. Board Can. 35:28-34.

Vollenweider, R.A. 1968. Water management research. OECD Paris. DAS/CS1/68.27. Mimeo. 183 p.

Nutrition and Air Quality

Alan L. Sutton
Department of Animal Sciences
Purdue University
West Lafayette, IN 47907
765-494-8012
asutton@purdue.edu

Summary

The pig's diet has a significant impact on the types and amounts of nutrients excreted and consequently the precursors of gas and odor emissions. Modifying the pig's diet can alter these gaseous emissions and impact air quality. Examples are presented on manipulating the pig's diet to alter nutrient excretion, emissions of ammonia, hydrogen sulfide and other volatile sulfide compounds, selected volatile organic compounds and other odorants, and the olfactometry responses to these dietary changes. Reducing the crude protein level and supplementing with synthetic amino acids; addition of low levels (<10%) of fermentable nonstarch polysaccharides; and the addition of feed additives that alter pH have promise of reducing odors and gaseous emissions. Additional research is needed to determine the impact of sources of protein, fermentable carbohydrates, and their ratio in the diets to minimize excretion of gas and odorous compounds. Manipulation of the microflora in the pig's digestive tract shows indication of some influence on gas emissions, however, research in this area has not received as much attention. Techniques to increase the availability and retention of nutrients can reduce excretions of compounds commonly causing gas emissions. Research to fine-tune these altered diets with different genetic lines under commercial production conditions is needed to assure consistent results and for encouraging implementation. Economics is still a major issue determining if certain manipulation technologies will be adopted.

Introduction

Manure is primarily a mixture of urine and feces excreted from pigs that contains undigested dietary components, endogenous end products, excreted excess absorbed nutrients, and indigenous bacteria from the lower gastrointestinal tract (GIT) of the pig. Manure contains a variety of organic compounds, complex to simple in nature, inorganic compounds, and potentially feed additives depending upon the makeup of the diet. Immediately after excretion and during extensive storage, anaerobic microbial degradation of manure often creates gaseous emissions and often times offensive odors, which have a negative impact on air quality. Numerous compounds (ranging from 160 to more than 400) have been identified from the anaerobic degradation of livestock manures. They have been generally grouped as volatile amines, sulfides, disulfides, organic acids, phenols, alcohols, carbonyls, nitrogen heterocycles, esters, fixed gases and mercaptans (Miner, 1975). In a review, Le, et al., (2004) grouped the volatile compounds in 4 groups based upon the origin. The groups were sulfurous compounds; indoles and phenols; volatile fatty acids (VFA); and ammonia and volatile amines.

Volatile fatty acids are predominantly acetic acid (60%) with propionic, butyric, (n-butyric), 2-methyl propionic (isobutyric), 3-methylbutyric (isovaleric), pentanoic (n-valeric) and capric acids in the order of decreasing concentrations. Much of the VFA come from the conversion of a multitude of fermentable carbohydrates (predominantly fiber fractions) and protein residues by indigenous bacteria in the large intestine and manure under anaerobic conditions. Common carbohydrate sources are cellulose, hemicellulose, non-starch polysaccharides and lignin (Le, et al., 2004). Straight-chain VFAs are produced from carbohydrate metabolism. In addition, acetic, propionic and butyric acids are produced from the deamination of amino acids such a L-glutamate, L-lysine and L-alanine (Le, et al., 2004). Branched chain VFA isovaleric acid and isobutyric acid can originate from the breakdown of peptides. Peptolytic bacteria

hydrolyze proteins into amino acids and the amino acids are deaminated and decarboxylated to the branch chain VFA. These VFA can be absorbed in the large intestine and contribute as an energy source for the pig.

Phenols, p-cresol, 3-methyl phenol (m-cresol) and 4-ethyl-phenol are predominant phenolic compounds and skatole and indole are the predominant indolic compounds commonly found in ventilation air from pig buildings (Le, et al., 2004). Most of the phenolic compounds are from the degradation of proteins. Key amino acids responsible for phenolic compounds are L-tyrosine. Indolic compounds are a result of microbial fermentation of L-tryptophan. Indolic and phenolic compound excretion can be directly from feces after the conversion of amino acids or indirectly from urine after the liver detoxifies the compounds by conjugation with glucuronic acid to form glucuronides. After excretion, β-glucuronidase present in feces will liberate the phenols and indoles in the manure mixture.

Sulfurous compounds can be produced from sulfate reduction and the metabolism of sulfur containing amino acids. Methionine, cysteine and cystine can be broken down to release sulfide compounds. When energy is limited, anaerobic bacteria can utilize these sulfur containing amino acids as a carbon and energy source. If sulfates are present, bacteria may use the sulfate as an electron donor for anaerobic respiration or if there is excess S, biosynthesis of cysteine and methionine can occur. This respiration process can create significant amounts of odor (Le, et al., 2004). In addition, hydrogen sulfide can be transformed to many other sulfide compounds (i.e., carbon disulfide, carbonyl sulfide) which can create distinct pungent odors.

The release of ammonia (NH_3) and volatile amines are the primary volatile nitrogenous compounds released from manure. Ammonia is the result of the deamination of amino acids when proteins are used as an energy source and the release of ammonia (NH_3) in the urine from the enzymatic conversion of urea by urease from the manure, which can occur within a short time after excretion. Volatile amines are created from the decarboxylation of amino acids and from the amination of aldehydes and demethylation of choline in the urine (Le, et al., 2004). Ammonia and sulfides can form in the atmosphere to create particulates which can be precipitated on the landscape in the form of ammonium sulfate and is considered as one of the compounds responsible for acid rain. Concern with acid rain includes the acidification of soils and the defoliate problem of plants.

Other gas emissions from swine production that are not odorous but can affect the atmosphere as greenhouse gases include methane and carbon dioxide. Methane is from anaerobic fermentation of carbohydrates and is commonly produced from manure storage systems. Carbon dioxide is from animal respiration and the decarboxylation of carbohydrates and proteins. Nitrous oxides are also contributors to the greenhouse gas problem, however, there is virtually no existence of nitrous oxides measured from pig buildings, however, the precursors of nitrous oxides (nitrogen compounds), which are numerous, can be converted to NO_x in certain situations.

Hobbs, et al. (1995) identified fifteen specific volatile organic compounds (VOC) as primary odor causing compounds from manures with available odor thresholds shown in Table 1. O'Neill and Phillips (1992) listed thirty compounds out of 168 compounds identified with an odor detection threshold of 1 $\mu g/m^3$ or less. The sensitivity of individual compounds by olfactometry threshold detection varies widely. In general, sulfur-containing compounds are among the group of lowest detection thresholds from manure. A major challenge for identifying specific odorous compounds from manures is that analytically derived gas concentrations and olfactometry measurements are not highly related (Gralapp, et. al, 2001; Van Kempen, et al., 2002). Methods that have been tested are GC-MS, electronic nose, scentometers, diffusion tubes, Fourier transform infrared spectrometry (FTIR) and olfactometry with the FTIR showing the most promise (Gralapp, et. al, 2001; Van Kempen, et al., 2002). Moreover, there are confounding effects of mixtures of different compounds on olfactometry detection. Some VOC can change chemical form, thus, precise analytical detection and quantification of air samples is also a challenge. A National Research Council Ad Hoc Committee on Air Emissions from Animal Feeding Operations determined that there is insufficient standardization of methodology for measurement of air quality pollutants from animal production operations and a lack of baseline data to be able to establish air quality standards for animal agriculture (NRC, 2003). As a result, a major research effort is underway to correct this deficiency.

Since the emission of volatile compounds to the air is directly related to the composition of nutrients from the diet that the bacteria have available, the

conditions at the time of microbial metabolism and the types of microorganisms during the degradation process, three basic approaches have been suggested for controlling gas emissions from animal production operations. One approach is to provide the pig, as closely as possible, with essential available nutrients based upon its genetic potential for lean tissue deposition and stage of growth, so that nutrient excretion is minimal and a lower potential for creating compounds responsible for gas emissions and odor production. Another concept is to manipulate the bacteria in the GIT of the pig by inhibiting certain microbial groups or altering the fermentation patterns of existing bacteria through diet manipulation, thus, controlling some gaseous emissions and odorous end products. Finally, changing the physical characteristics of urine and feces by diet manipulation may change conditions to inhibit some gas emissions.

Improving Feed Efficiency.

Any management practice that improves the overall efficiency of feed utilization in a swine herd will generally reduce the total amount of manure produced, should reduce nutrient excretion and potentially the precursors of volatile producing compounds. Using growth promoters such as antibiotics, β-agonists, and maintaining a high health status are examples of how one can improve feed conversion efficiency and reduce nutrient excretion. Use of therapeutic levels of feed grade antibiotics can improve feed efficiency from 5 to 15% and have reduced some isolated odor compounds (p-cresol; skatole). Copper sulfate addition to the diet has been shown to improve feed efficiency of 5 to 10% and has been shown to potentially reduce odors (Armstrong, et al., 2000).

Two experiments were conducted with crossbred barrows (Initial BW=84 kg) comparing the effects of a control corn-soybean meal diet and low crude protein diets with and without a β-agonist, ractopamine (RAC), on N and P retention and excretion, and gas and odor emissions (Sutton, et al, 2001). The four dietary treatments tested were: 1) 13.8% CP, 0.8% Lys., control; 2) 16.1% CP, 1.1% Lys.; 3) 16.1% CP, 1.1% Lys. + 20 ppm RAC; and 4) 13.8% CP, 1.1% Lys. + 20 ppm RAC. Synthetic amino acids added to the diets were (Diet 1) L-lysine-HCl (0.17%); (Diets 2 and 3) L-lysine-HCl (0.38%), DL-methionine (0.06%), L-threonine (0.12%) and L-tryptophan (0.01%); and (Diet 4) L-lysine-HCl (0.62%), DL-methionine (0.10%), L-threonine (0.23%), L-tryptophan (0.05%), L-isoleucine (0.11%) and L-valine (0.15%). Averaged across both experiments (Tables 2 and 3), the 16.1% CP diet with RAC decreased total N excretion by 10.7% or 2.49 g/d ($P < 0.05$) and total manure output by 3.9% or 149 g/day. The low crude protein diet (13.8%) with RAC further decreased N excretion by 34.2% or 7.65 g/d ($P<0.05$). Slurry ammonium N (Table 4) was reduced 8-21% and 21-47% ($P<0.05$) from pigs fed RAC at 16.1% and 13.8% dietary CP, respectively, compared to similar CP diets without RAC. Total VFA production (Table 5) was higher ($P<0.05$) for the 16.1% CP diet with no RAC compared to the diets with RAC and was 21%, 28% and 23% higher ($P<0.05$) at d 17, 35 and 64 of the trial, respectively, than the 13.8% CP diet + RAC. Olfactometry results (Table 6) indicated a detection threshold 51% higher ($P<0.05$) for the 16.1% CP diet without RAC compared to the 13.8% CP diet without RAC at d 64. This indicated that a 13.8% CP diet + 20 ppm RAC could significantly decrease slurry ammonium N and VFA production in stored manure to help reduce ammonia and odor emissions. Research has not been conducted with RAC at lower inclusion levels in the pig's diet and with commercial type settings.

Other feed management practices that enhances feed efficiency and reduces nutrient excretion are: fine grinding, pelleting and other feed processing techniques, reduced feed wastage, dividing the growth period into more phases (phase feeding) with less spread in weight between groups allows producers to more closely meet the pig's protein and other nutrient requirements, and split-sex feeding to meet the specific requirements of gender (Lewis and Southern, 2001). However, the effects of these management technologies on gas emissions have not been investigated.

Often, a safety margin for diet ingredients is allowed so that variability of nutrients in feed ingredients and variation in pig biological responses can be reduced. Formulating the diets to meet the protein/amino acid requirements of pigs (National Research Council; NRC, 1998) without excessive levels above the requirements, however, is critical. Feeding excessive protein levels is not only wasteful and expensive, but it results in excessive N excretion and increased ammonia emission (Sutton, et al., 1999).

Nitrogen Manipulation

Fecal N arises from undigested dietary protein, intestinal secretions (mucin, enzymes, etc.), sloughed intestinal cells, and intestinal bacteria. Urinary N, largely in the form of urea, arises from the breakdown of absorbed dietary amino acids that are in excess of the amounts needed for lean tissue protein synthesis, and from the normal breakdown/turnover of body tissue proteins. Typical commercial diets are corn/soybean meal based and do not contain optimal ratios or availabilities of amino acids for lean tissue protein synthesis and maintenance. Consequently, intact protein sources are added to the diet to meet the most limiting amino acid (usually lysine) but other amino acids are in excess. The excess amino acids are used for energy and the N is excreted as urea in the urine. Today, if economics will allow, nutritionists are formulating swine feeds on an "ideal protein" basis using synthetic amino acids where the available amino acid concentrations can be added to more closely match those needed by the pig for lean tissue protein synthesis and maintenance.

Several studies in Europe and the US have shown that with a 1% reduction in the crude protein of a pig diet and supplementation with limiting synthetic amino acids will reduce ammonia emission into the air by 8 to 12% (Kerr and Easter, 1995; Kendall, et al., 1998; Sutton, et al., 1999). Practical feeding studies with CP levels reduced from 19% to 13% resulted in a 47% to 59% reduced NH_3 emissions from building air. This reduction in dietary N also reduced manure odors by 40 to 86% and decreased p-cresol by 43% (Hobbs et al., 1996). Turner, et al., (1996), showed reduced ammonia emissions from manure in a pilot study of 79% by reducing the dietary CP level in the feed from 16% to 12% plus synthetic amino acids during the growing phase and of 58% when reducing the dietary CP level in feed from 14% to 10% plus synthetic amino acids during the finishing phase. Using model pits, Otto, et al., (2003) showed a decrease in ammonia emissions when reducing the CP content of the semi-synthetic diets from 15% to 0%. However, there was an increase in fecal VFA concentrations but not phenols in urine and there was no change in odor offensiveness when the CP contents of the diets were reduced.

Kendall, et al., (1998) fed a reduced crude protein (RCP) (12.2% CP) corn-soy diet with synthetic lysine (0.41%), methionine (0.013%), tryptophan (0.03%), and threonine (0.039%) to 27 kg pigs for nine weeks and compared to pigs fed a high crude protein (HCP) corn-soy diet (16.7% CP). Slurry manure contents had a lower pH (.4 units), lower total N (40%) and lower ammonium N (20%) from pigs fed the RCP diet compared to the slurry manure from pigs fed the HCP diet (Table 7). A 40% reduction of aerial ammonia and hydrogen sulfide occurred in the rooms housing the RCP pigs (Table 8). In addition, total odors were reduced by 30% based upon olfactometry analysis of room air. However, growth rate and feed efficiency of the pigs fed the RCP diet was reduced by 4 to 5% compared to the pigs fed the HCP diet. Back fat depth was also increased slightly (0.13 cm) in the pigs fed the RCP diet.

In another research study, (Kendall, et al., 1999), 10% soybean hulls were added to the reduced crude protein (RCPF) diet and compared to the normal crude protein (HCP) diet. In this study, 60 kg pigs were fed either a HCP diet (12.4% CP) or a RCPF diet (9.7% CP) with fiber. The RCPF diet was supplemented with 0.372% lysine, 0.005% tryptophan and 0.042% threonine. As in the previous study, the slurry manure contents were decreased in pH (.3 units), total N (23%) and ammonium N (29%). Ammonia N was decreased by 40% and hydrogen sulfide was reduced by 26.5% in the air of the room housing the pigs fed the RCPF diet with fiber compared to the room housing the pigs fed the HCP diet (Table 9). In two replicate trials, the pig growths were similar between the two diets. In two additional replicate trials, pigs fed the HCP diets gained faster than the pigs fed the RCPF diet, principally related to feed intake being reduced by 6% in pigs fed the RCPF diet. However, in a subsequent study, when additional synthetic amino acids were added to the diet, gilts performed similar to those gilts fed the HCP diet including carcass quality and yield. This indicates that there may have been too great of a dietary reduction in amino acids in the previous trials.

A group feeding experiment was conducted by Hill, et al, (2001) with 200 grow-finish pigs (initial BW=92.3 kg) to further evaluate dietary CP reduction of swine diets to reduce aerial pollutants and nutrient excretion. The diets consisted of either a control, corn-soybean diet (13.1% CP, 0.52% true ileal digestible lysine for the barrows and 14.2% CP, 0.59% true ileal digestible lysine for the gilts) or a reduced CP diet with supplemental synthetic amino acids (9.7% CP, 0.52% true ileal digestible lysine for the barrows and 10.6% CP, 0.59% true ileal digestible lysine for the gilts). Aerial ammonia concentration, hydrogen sulfide, and detection threshold of odor samples were

taken at wk 2 and 4 from both room and exhaust air. Pigs fed control or low crude protein diets had similar overall average daily gain (790 vs. 784 g/d), overall gain:feed (0.304 vs. 0.297), and average daily feed intake (2601 vs. 2649 g/d). Pigs fed low CP diet had greater wk 4 loin depth (57 vs. 55 mm; P<0.09), numerically higher backfat thickness (17.9 vs. 16.9 mm) and greater total loin depth increase (5.0 vs. 3.1 mm; P<00.02). By wk 4, there was a 60.4% reduction in aerial ammonia concentration (P<0.04) from room air and 52.2% reduction in the exhaust air ammonia concentration (13.4 vs 28.1 ppm; P<0.0003) when pigs were fed low CP diets. At wk 4, the stored manure from pigs fed low CP diets had 29.8% less total N (P< 0.0001), 30.6% lower ammonium N (P<0.0001), 35.8% less total N accumulation in the manure (P<0.01), and a lower manure pH (7.25 vs. 7.61; P<0.0001). It is clear from these studies that amino acid balanced (correct ratios and concentrations) diets with lower intact CP levels are effective at reducing aerial ammonia, manure N, and manure pH. In addition, if adequate amino acid levels are included in the diet, growth performance is comparable to a diet without synthetic amino acids.

Fermentable Carbohydrate

The types and amounts of fermentable carbohydrates in the pig's diet influences the emissions of VFA, ammonia and indolic compounds. One can reduce the emission of ammonia by altering the ratio of N excretion in urine and feces with the addition of fermentable carbohydrates. Complex carbohydrates such as β-glucans, and other non-starch polysaccharides (NSP) can influence endogenous N excretion at the terminal ileum and enhance microbial fermentation in the large colon resulting in increased bacterial protein production. Microbial metabolism and production is enhanced through providing more energy to the bacteria and using available N for protein synthesis, which will reduce the N excretion in urine as urea and shifting the N excretion more into the feces in the form of bacterial protein. The end result is that ammonia volatilization is reduced and the pH of the urine reduced because less urea is being excreted. Examples are manure from pigs fed sugar beet pulp and soybean hulls in the pig's diet reduced ammonia emissions, pH of urine, feces and slurry, and changed the relative level of N excretion in feces and urine.

Growing barrows were fed low CP diets with synthetic amino acids and two sources of fiber to determine odors and nutrients from fresh manure and manure stored in model anaerobic manure storage systems (Hankins, et al., 1998). Dietary treatments included: (1) Diet I: a standard 15% crude protein corn-soy diet with 0.75% total lysine; (2) Diet II: a 11% crude protein corn-soy diet with 0.39% crystalline lysine (0.76% total Lys), 0.05% methionine (0.25% total Met), 0.05% tryptophan (0.15% total Trp) and 0.11% threonine (0.51% total Thr) added; (3) Diet III: Diet II with 10% soybean hulls (SH), or (4) Diet IV: Diet II with 10% dried sugar beet pulp (SBP).

The lower CP diet with and without the addition of fiber reduced the pH of fresh manure (Table 10). Ammonia N in fresh manure was decreased 31%, 55% and 47% with the lower CP, lower CP plus SH or dried SBP diets, respectively. There was 35% less ammonia N excretion in fresh manure when the reduced CP diet was fed with SH as a fiber source as compared to the reduced CP diet alone. There was a 38%, 50% and 42% reduction in total N excretion in fresh manure with the lower CP, the lower CP plus soybean hulls and the lower CP plus dried SBP diets, respectively. There were no significant effect on VFA in fresh manure; however, there was a trend towards more acetic acid and total VFA production in fresh manure when fiber was added to the diet (Table 11). Similar responses were observed in a similar companion study with SH and SBP by Shriver, et al. (2002).

Similar treatment effects were observed with anaerobically stored manure. There was a significant reduction in ammonia and total N with the lower CP diet and synthetic amino acids either alone (37% and 26% reduction for ammonia and 40% to 29% reduction for total N on a wet and DMB, respectively) or with 10% soybean hulls (41% and 39% reduction for ammonia and 34 % and 32% reduction for total N on a wet and DMB, respectively) or 10% dried SBP (62% and 64% reduction for ammonia and 43 % and 46% reduction for total N on a wet and DMB, respectively) (Table 10). In addition, on a dry matter basis, SBP further reduced ammonia N compared to the other treatments. VFA tended to be lower in stored manure from pigs fed the SBP diet compared to the other diets (Table 11). The addition of SH and SBP reduced benzene, dimethyl disulfide, 2,2-dimethyl hexane and hexane in stored manure. The lower CP diet also reduced the previously stated VOC in stored manure as well as methyl pentane.

In a group feeding study, 150 grow-finish pigs (initial BW=85.3 kg) were used to evaluate the inclu-

sion of soybean hulls in swine diets to reduce aerial pollutants and alter manure composition (DeCamp, et al., 2001). Diets were split-sex fed and consisted of either a corn-soybean meal based control (C) or the control diet with the addition of 10% soybean hulls (SH) and 3.4% supplemental fat (all diets=3370 Kcal ME/kg; barrows 12.0% CP, 0.53% dLys; gilts 12.7% CP, 0.57% dLys).

Pigs fed SH diets had greater overall ADG (905 vs 859 g/d; P<0.03) and tended to have higher G: F (0.326 vs 0.310; P<0.09) with no difference in ADFI. Pigs fed SH had greater adjusted backfat (113 kg BW) at wk 6 (15.8 vs 14.7 mm; P<0.001) than C pigs. There was a 20% reduction in aerial ammonia concentrations (P<0.02), a 32% reduction in hydrogen sulfide (P<0.003) and an 11% reduction in odor detection threshold when pigs were fed SH diets. Individual manure VFA concentrations were increased, with total manure VFA concentrations increasing by 32% (P<0.001) when pigs were fed SH (Table 13). The stored manure from pigs fed SH diets had 21% greater total N (22.5 vs 18.6 kg; P<0.02), an 8% increase in ammonium N (18.3 vs 16.9 kg; P<0.05) and a decreased manure pH (7.12 vs 7.26; P<0.03) (Table 14). This increased manure N should be in a more stable microbial protein form with less volatile emissions and provide a reduced environmental runoff potential. However, the impact was greater when low CP and synthetic amino acid diets included fiber versus no CP reduction.

Shurson et al. (1999) stated that feeding nursery pigs can potentially reduce hydrogen sulfide and other odors from nursery facilities. In an attempt to use a combination of several dietary techniques, Kendall, et al. (2001) conducted a study with one-hundred and eighty grow-finish pigs. In the first experiment, diets consisted of either a control (C), corn-soybean diet (11.5% CP, 0.60% Lysine; Lys) or a reduced CP diet formulated with high-available phosphorus corn, 272 units of phytase, 5% soybean hulls and a reduced mineral sulfate trace mineral premix (8.25% CP, 0.57% Lys; HRP) with supplemental synthetic amino acids. In the second experiment, barrows were fed the same diets as the first experiment, but two additional diets were formulated for gilts; control (12.6% CP, 0.63% Lys) and HRP (9.35% CP, 0.60% Lys) with supplemental synthetic amino acids. All diets were isocaloric and equal in digestible Lys. Pigs fed control diets in the first experiment were heavier, had higher average daily gain (824 vs. 735 g/d; P<0.004), were more efficient, had greater loin depths at week 6, and less accumulation of backfat. In contrast, the pigs fed control diets in the second experiment were nearly identical to pigs fed HRP diets in every respect. By week 6, there was a 48.7% reduction in aerial ammonia concentrations (P<0.03) from room air and 49.8% reduction in the exhaust air (P<0.04) for pigs fed HRP diets. Hydrogen sulfide levels were 48% lower and detention threshold was 37% lower at week 6 in room air where HRP diets were fed. At week 6, the stored manure from pigs fed RCP diets had 26.9% less total-N, 29.5% lower ammonium-N, 51.7% less excreted P and had a lower pH. The manipulation of the HRP diet was successful at reducing aerial ammonia concentrations, hydrogen sulfide, detection threshold, manure N, P and pH with comparable performance in the pigs as long as adequate amino acid levels were included in the diet.

Hankins, et al., (2001) combined several diet strategies together in an attempt to reduce nutrient excretions and ammonia emissions from growing pigs. A standard 13.1% CP corn-soybean meal based diet with 0.23% available P (CTL) was compared to a 11.5% CP diet with 0.15% lysine-HCl and 0.26% available P (AA) and an 8.25% CP diet with 5% soybean hulls, high available corn (HAP), 0.05% phytase, reduced mineral sulfates, 0.40% lysine-HCl and 0.16% available P (HRP). Total N excretion was reduced by 37% and 55% for the AA and HRP diets, respectively. Total P excretion was reduced by 63% with the HRP diet with the water soluble P reduced by 41%. During manure storage, manure slurry from the HRP fed pigs increased VFA by 41% and 37% at d 28 and 43 of the trial, respectively. Ammonia emissions were reduced by 15.5% and 63.2% with the AA and HRP diets, respectively compared to CTL at d 28 and reduced by 11.9% and 59.7% with the AA and HRP, respectively compared to CTL at d 43 of the trial.

Microbial Manipulation

Attempts have been made to isolate and identify the microbial populations in the digestive systems of pigs that control or are involved in the creation of odors. Reviews by Mackie, et al., 1998 and Le, et al., 2004 have summarized known bacterial species capable of inhabiting the GIT of pigs and their role in gas and odor emissions. A few examples of means to alter microbial fermentation patterns and populations are illustrated here. Mackie, et al., (1998) stated that the bacterial genera involved with deamination of

amino acids were *Bacteroides, Prevotella, Selenomonas, Butyrivibrio, Lachnospira, Eubactreium, Fusobacterium, Clostridium, Peptostreptococcus, and Acidaminococcus*. The production of indoles and phenols was primarily from microbial metabolism of amino acids. Ward et al. (1987) isolated an obligate anaerobe of the Lactobacillus sp. that decaboxylated p-hydroxyphenylacetic acid to 4-methylphenol (p-cresol) in swine feces. Yokoyama and Carlson, (1979) reported that several *Clostridia* sp., *E. coli* and *Bacteroides thetaiotaomicron* can be involved with indole and skatole production. Compounds such as oligosaccharides (fructooligosaccharides, mannoligosaccharides, sucrose thermal oligosaccharide carmel, inulin, arabinogalactan, galactan), dairy byproducts (lactulose, lactitol, lactose, whey), and organic acids (propionic, fumaric, citric) have been added to manipulate the microflora populations.

Fructooligosaccharides have been shown to alter VFA patterns in the lower GIT (reduce proportion of acetate and increase the proportion of propionate), reduce total aerobes, predominantly coliforms, increase bifidobacteria (Houdijk et al., 1999) and reduce odorous compounds from swine manure (Hidaka et al., 1986). Inulin fed at 5% a corn-soybean meal base diet to 30 kg pigs did not affect fecal excretion of VFA, ammonia, total volatile sulfides, p-cresol and indole (Rideout, et al., 2004). However, inulin in the pig's diet did reduce skatole concentrations in feces. In earlier work, antibiotics (chlortetracycline, sulfamethazine, penicillin) fed to pigs reduced urinary excretion of p-cresol (Yokoyama et al., 1982). Lincomycin sulfate did not affect p-cresol excretion. Recent work by Japanese researchers, have shown that tea polypheonols in swine diets lowered the production of ammonia, phenol, p-cresol, ethylphenol, indole and skatole in swine feces (Terada et al., 1993). Also, the tea polypheonols were shown to significantly reduce certain pathogenic species *(Mycoplasma pneumonia, Staphylococcus aureus,* and *Clostridium perfringens;* Hara and Ishigami, 1989; Chosa et al., 1992).

Physical Characteristics

Several studies have been conducted showing the effects of reducing the pH of manure and ammonia emission by adjusting the dietary electrolyte balance, reducing urea excretion or increased volatile fatty acid production. Canh et al. (1997) showed that increasing the NSP content and decreasing the electrolyte balance (dEB) of the diet reduced the pH of pig slurry. Inclusion of 30% sugar beet pulp (with 31.2% NSP) reduced the pH of slurry by 0.44 to 1.13 pH units lower than a by-product diet (with 18.2% NSP), grain-based diet (with 13.8% NSP) and a tapioca-based diet (with 13.5% NSP). The decreased dietary electrolyte balance (expressed as mEq Na + K - Cl) in the diet reduced the pH of urine and subsequent slurry. Canh et al. (1996) and Mroz et al. (1996) showed that dietary calcium salts and electrolyte balance significantly influenced urinary pH and subsequent pH and ammonia emission from pig slurry. Mroz et al. (1997) showed that increasing the levels of calcium benzoate (2, 4, 8 g/kg feed) in the diet of sows significantly reduced pH of urine from 7.7 to 5.5 and reduced ammonia emissions up to 53%. In nursery diets, Colina et al. (2001) observed a significant reduction in ammonia emissions in the rooms housing nursery pigs. However, feed intakes were lowered and performance was depressed when 1.96% calcium chloride was added to the diet. Van Kempen et al. (2001) showed the benefits of using adipic acid in pig diets to reduce pH and ammonia emissions but did not improve lysine utilization in the pig.

Bentonite and zeolite materials have been tested to bind ammonia in manure and reduce release of ammonia to the atmosphere. Kreiger et al. (1993) did not find any affect of feeding a naturally processed clinoptilolite on ammonia emissions from swine manure. Pilot studies in Canada showed a 21% reduction in ammonia N in the room of pigs fed 5% zeolite (tektosilicate). Zeolites can also absorb phenols. However, when these products are fed to the pig there has been little effect on manure odor. Sarsaponin extracts from the *Yucca schidigera* plant has been incorporated in the pig's diet as a growth promotant and to reduce ammonia emission from manure. Ammonia emission was significantly suppressed by 55.5% in fresh manure tested in an incubation trial from pigs fed the sarsaponin extract. However, Kemme et al. (1993) conducted incubation trials with manure and did not verify the same response to ammonia inhibition and found that 6000 mg/kg of the extract was necessary for maximal suppression of ammonia from urea. Recent work by Colina et al. (2001) has shown that addition of *Yucca schidigera* to nursery pig diets reduced ammonia emissions in nursery rooms.

Geisting and Easter (1986) summarized studies incorporating organic acids (citric, hydrochloric, propionic, fumaric, and sulfuric) at dietary levels of 1 to 4% showing variable results in pH effects on

digesta and growth effects on swine. Risely et al. (1992) also showed that addition of fumaric or citric acids (1.5%) had very little effect on pH, volatile fatty acids, or chlorine concentrations of intestinal contents of swine. There has been very little research on the effects of organic and inorganic acids on gas and odor emissions.

Implications

Modifying the pig's diet to reduce gas emissions and odors is feasible and practical. Reducing the crude protein level and supplementing with synthetic amino acids, addition of low levels (<10%) of fermentable nonstarch polysaccharides, and addition of pH and buffering feed ingredients have promise. Feed management practices of formulating diets to meet the lean tissue accretion levels of the specific genetic lines, phase feeding, split-sex feeding and minimizing feed wastage will assist in reducing nutrient excretions. Manipulation of the microflora in the pig's digestive tract shows indication of some influence on gas emissions; however, work in this area has not received as much research attention. Techniques to increase the availability and retention of nutrients can reduce excretions of compounds commonly causing odors. Implementation of several of these technologies and practices often in combinations have the potential of reducing ammonia and hydrogen sulfide emissions by 30 to 50% and reduce odors by 30% at very little cost to the producer. Research to fine-tune these altered diets with different genetic lines under commercial production conditions is needed to assure consistent results and for encouraging implementation. Economics is still a major issue determining if certain manipulation technologies will be adopted.

References

Armstrong, T. A., C. M. Williams, J. W. Spears and S. S. Shiffman. 2000. High dietary copper improves odor characteristics of swine waste. J. Anim. Sci. 78:859-864.

Canh, T. T., A. J. A. Aarnink, Z. Mroz, and A. W. Jongbloed. 1996. Influence of dietary calcium salts and electrolyte balance on the urinary pH, slurry pH and ammonia volatilization from slurry of growing-finishing pigs. ID-DLO Rept. 96-51. 39 pp.

Canh, T. T., M. W. A. Verstegen, A. J. A. Aarnink and J. W. Schrama. 1997. Influence of dietary factors on nitrogen partitioning and composition of urine and feces in fattening pigs. J. Anim. Sci. 75:700-706.

Chosa, H., M. Toada, S. Okubo, Y. Hara and T. Shimamura. 1992. Antimicrobial and microbicidal activities of tea and catechins against Mycoplasma. J. Japanese Assoc. Infectious Dis. 66:606-611.

Colina, J. J., A. J. Lewis, P. S. Miller and R. L. Fisher. 2001. Dietary manipulation to reduce aerial ammonia concentrations in nursery facilities. J. Anim. Sci. 79:3096-3103.

DeCamp, S. A., B. E. Hill, S.L. Hankins, D. C. Kendall, B.T. Richert, A.L. Sutton, D.T. Kelly, M.L. Cobb, D.W. Bundy, and W.J. Powers. 2001. Effects of soybean hulls in a commercial diet on pig performance, manure composition, and selected air quality parameters in swine facilities. J. Amim. Sci. 79 (Suppl. 1):252

Giesting, D. W. and R. A. Easter. 1985. Response to starter pigs supplementation of corn soybean meal diets with organic acids. J. Anim. Sci. 60:1288

Geisting, D. W. and R. A. Easter. 1986. Acidification status in swine diets. Feed Management. 37:8, 10, 53.

Gralapp, A. K., W. J. Powers, D. S. Bundy. 2001. Comparison of olfactometry, gas chromatography, and electronic nose technology for measurement of indoor air from swine facilities. Trans. ASAE. 44:1283-1290.

Hankins, S.L., A.L. Sutton, J.A. Patterson, B.T. Richert, A.J. Heber, D.T. Kelly, K.B. Kephart, R. Mumma, E. Bogus, and S.D. Carter. 2001. Reduction in odorous compounds in pig manure through dietary fiber manipulation. J. Anim. Sci. 79:60.

DeCamp, S. A., B. Hill, S. L. Hankins, C. T. Herr, B. T. Richert, A. L. Sutton, D. T. Kelly, M. L Cobb, D. W. Bundy and W. J. Powers. 2001. Effects of soybean hulls on pig performance, manure composition and air quality. Purdue Swine Res. Report pp. 84-89.

Hankins, S. L., D. C. Kendall, S. A. DeCamp, D. T. Kelly, A. L. Sutton and B. T. Richert. 2001a. Effect of several known dietary manipulations used in combination on nutrient concentration and ammonia emission of stored manure. Purdue Swine Res. Report pp. 90-96.

Hankins, S. L., D. C. Kendall, S. A. DeCamp, D. T. Kelly, A. L. Sutton and B. T. Richert. 2001b. Digestibility of several known dietary manipulations used in combination to reduce nutrient excretion in pigs. Purdue Swine Res. Report pp. 103-111.

Hara, Y. and T. Ishigami. 1989. Antibacterial activities of polyphenols against food borne pathogenic bacteria. Nipppon Shokuhin Gakkaishi 36:996-999.

Hidaka, H., T. Eida, T. Takizawa, T. tokanaga and Y. Tashiro. 1986. Effect of fructooligosacchairdes on intestinal flora and human health. Bifidobact. Microflora 5:37-50.

Hill, B. E., S. L. Hankins, D. C. Kendall, B. T. Richert, A. L. Sutton, D. T. Kelly, M. L. Cobb, D. W. Bundy and W. J. Powers. 2001. Effects of soybean hulls in a commercial diet on pig performance, manure composition, and selected air quality parameters in swine facilities. J. Anim. Sci. 79:252. (Abstract)

Hobbs, P. J., T. H. Misselbrook and B. F. Pain. 1995. Assessment of odours from livestock wastes by photoionization detector, an electronic nose, olfactometry and gas chromatography-mass spectorometry. J. Agric. Eng. Res. 60:137-144.

Hobbs, P. J., B. F. Pain, R. M. Kay and P. A. Lee. 1996. Reduction of odorous compounds in fresh pig slurry by dietary control of crude protein. J. Sci. Food Agric. 71:508-514.

Houdijk, J. G. M., M. W. Bosch, S. Tamminga, M. W. A. Verstegen, E. B. Berenpas and H. Knoop. 1999. Apparent ileal and total tract nutrient digestion by pigs as affected by dietary nondigestible oligosaccharides. J. Anim. Sci. 77:148-158.

Kemme, P. A., A. W. Jongbloed, B. M. Dellaert and F. Krol-Kramer. 1993. The use of a Yucca Schidigera exctract as 'urease inhibitor' in pig slurry. Proc. First Inter. Symp. Nitrogen Flow in Pig Production and Environ. Consequences. pp. 330-335. Wageningen, NL

Kendall, D. C., K. M. Lemenager, B. T. Richert, A L. Sutton, J. W. Frank and B. A. Belstra. 1998. Effects of intact protein diets versus reduced crude protein diets supplemented with synthetic amino acids on pig performance and ammonia levels in swine buildings. J. Anim. Sci. 76(Suppl. 1):173. (Abstract)

Kendall, D. C., B. T. Richert, A. L. Sutton, J. W. Frank, S. A.DeCamp, K. A. Bowers, D. Kelly, M Cobb. 1999. Effects of fiber addition (10% soybean hulls) to a reduced crude protein diet supplemented with synthetic amino acids verses a standard commercial diet on pig performance, pit composition, odor and ammonia levels in swine buildings. J. Anim. Sci. 77 (Suppl):176 (Abstract)

Kendall, D. C., B. T. Richert, K. A. Bowers, S. A. DeCamp, C.T. Herr, T. E. Weber, M. Cobb, D. Kelly , A. L. Sutton, D. W. Bundy, and W. J. Powers. 2001. Effects of Dietary Manipulation on Pig Performance, Manure Composition, Aerial Ammonia, Hydrogen Sulfide, and Odor Levels in Swine Buildings. J. Anim. Sci. 79 (Suppl): 59 (Abstract)

Kerr, B. J. and R. A. Easter. 1995. Effect of feeding reduced protein, amino acid-supplemented diets on nitrogen and energy balance in grower pigs. J. Anim. Sci. 73:3000-3008.

Kreiger, R. J. Hartung and A. Pfeiffer. 1993. Experiments with a feed additive to reduce ammonia emissions from pig fattening housing – preliminary results. Proceedings of the First International Symposium. Nitrogen Flow in Pig Productin and Environmental Consequences. Wageningen, Netherlands. June 8-11. pp. 295-300.

Le, P. D., A. J. A. Aarnink, N. W. M. Ogink, P. M. Becker and M. W. A. Verstegen. 2004. Odour from animal production facilities: its relationship to diet. Nutr. Res. Rev. 17: In press.

Lewis, A. J. and L. Southern, L. L., Ed. 2001. Swine Nutrition. 2nd edition. CRC Press. Boca Raton, FL.

Miner, J. R. 1975. Management of odors associated with livestock production. Proc. 3rd Inter. Symp. Livest. Waste, ASAE., pp. 378-380.

Mroz, Z., A. W. Jongbloed, K. Vreman, T. T. Canh, J. Th. M. van Diepen, P. A. Kemme, J. Kogut and A. J. A. Aarnink. 1996. The effect of different cation-anion supplies on excreta composition and nutrient balance in growing pigs. ID-DLO Rep. 96.028.

Mroz, Z., W. Krasucki and E. Grela. 1997. Prevention of bacteriuria and ammonia emission by adding sodium benzoate to diets for pregnant sows. Ann. Mtg. EAAP. Vienna, Austria. August 25-28.

National Research Council. 1998. Nutrient Requirements of swine. 10th rev. ed. National Academy Press, Washington, DC.

National Research Council. 2003. Air emissions from animal feeding operations: current knowledge, future needs. Ad hoc committee on Air Emissions from Animal Feeding Operations, Committee on Animal Nutrition. 286 pages.

O'Neill, D. H. and V.R. A. Phillips. 1992. Review of the control of odour nuisance from livestock buildings. Part 3: properties of the odorous substances which have been identified in livestock wastes or in the air around them. J. Agric. Eng. Res. 53:23-50.

Otto, E. R., M. Yokoyama, S. Hengemuehle, R. D. von Bernuth, T. van Kempen and N. L. Trottier. 2003. Ammonia, volatile fatty acids, phenolics, and odor offensiveness in manure from growing pigs fed diets reduced in protein concentration. J. Anim. Sci. 81:1754-1763.

Rideout, T. C., M. Z. Fan, J. P. Cant, C. Wagner-Riddle and P. Stonehouse. 2004. Excretion of major odor-causing and acidifying compounds in response to dietary supplementation of chicory inulin in growing pigs. J. Anim. Sci. 82:1678-1684.

Risley, C. R., E. T. Kornegay, J. D. Lindemann, C. M. Wood and W. N. Eigel. 1992. Effect of feeding organic acids on selected intestinal content measurements at varying times postweaning pigs. J. Anim. Sci. 70(1): 196-206.

Shriver, J. A., S. D. Carter, A. L. Sutton, B. T. Richert, B. W. Senne and L. A. Pettey. 2002. Effects of adding fiber sources to reduced crude protein, amino acid-supplemented diet on nitrogen excretion, growth performance, and carcass traits in finishing pigs. J. Anim. Sci. (In press).

Shurson, J., M. Whitney and R. Nicolai. 1999. Manipulating diets may reduce hydrogen sulfide emissions. Feedstuffs. 25 January:12-17.

Sutton, A. L., K. B. Kephart, J. A. Patterson, D. T. Kelly, E. Bogus, D. D. Jones, A.J. Heber and J. Nielsen. 1998a. Manipulating Nitrogen in Pig Diets to Reduce Manure Nitrogen Excretion and Odors. Swine Day Report 147-155.

Sutton, A. L., J. A. Patterson, O. L. Adeola, B. T. Richert, D. T. Kelly, J. Nielsen, A.J. Heber, K. B. Kephart and E. Bogus. 1998b. Amino Acid and Mineral Manipulation in Pig Diets to Reduce Nitrogen and Odors in Pig Manure. Swine Day Report 165-174.

Sutton, A. L., K. B. Kephart, M. W. A. Verstegen, T. T. Canh and P. J. Hobbs. 1999. Potential for reduction of odorous compounds in swine manure through diet modification. J. Anim. Sci. 77:430-439.

Sutton, A. L., B. T. Richert, S. L. Hankins, S. A. DeCamp and A. L. Carroll. 2001. Potential impact of ractopamine on environmental stewardship. J. Anim. Sci. 79:238.

Terada, A., H. Hara, S. Nakajyo, H. Ishikawa, Y. Hara, K. Kukai, K. Kobayashi and T. Mitsouka. 1993. Effect of supplemetns of tea polyphenols of the caecal flora and caecal metabolites of chicks. Microb. Ecol. Health and Dis. 6:3-9.

Turner, L. W., G. L.Cromwell, T. C. Bridges, S. Carter and R. S. Gates. 1996. Ammonia (NH_3) emission from swine waste as influenced by diet manipulation. Proc. *Ist Inter. Conf. Air Pollution from Agr. Operations*. Pp. 453-458. Kansas City, Missouri. February 7-9.

Van Kempen, T. A. T. G. 2001a. Dietary adipic acid reduces ammonia emission from swine excreta. J. Anim. Sci. 79:2412-2417.

Van Kempen, T. A. T. G., W. J. Powers and A. L. Sutton. 2002. Technical note: Fourier transform infrafred (FTIR) spectroscopy as an optical nose for predicting odor sensation. J. Anim. Sci. 80:1524-1527.

Ward, L. A., K. A. Johnson, I. M. Robinson and M. T. Yokoyama. 1987. Isolation from swine feces of a bacterium which decarboxylates p-hydroxyphenylacetic acid to 4-methylphenol (p-cresol). Appl. Environ. Micro. 53:189-192.

Yokoyama, M. T. and J. R. Carlson. 1979. Microbial metabolites of tryptophan in the intestinal tract with special reference to skatole. Am. J. Clin. Nutr. 32:173-178.

Yokoyama, M. T., C. Tabori, E. R. Miller and M. G. Hogberg. 1982. The effects of antibiotics in the weanling pig diet on growth and the excretion of volatile phenolic and aromatic bacterial metabolites. Am. J. Clin. Nutr. 34:1417-1424

Table 1. Odour detection thresholds for odorants present in pig slurry[a]

Compound	Odour detection threshold ($mg\ m^{-3}$)
Acetic acid	25-10,000
Propanoic acid	3-890
Butanoic	4-3000
3-Methyl butanoic acid	5
Pentanoic acid	.8-70
Phenol	22-4000
4-Methyl phenol	.22-35
Indole	.6
3-Methyl indole	.4-.8
Methanethiol	.5
Dimethyl sulfide	2-30
Dimethyl disulfide	3-14
Dimethyl trisulfide	7.3
Hydrogen sulfide	.1-180

[a] Hobbs, et al. (1997)

Table 2. Nitrogen Excretion (Trial 1)*.

Dietary CP	13.8% CP		16.1% CP		
Ractopamine, 20 ppm	-	+	-	+	CV
Water Intake, mL	4800	4800	4800	4800	--
Feed Intake, g/d	2200	2200	2200	2200	--
Feces, g/d, as is	485[a]	654[b]	578[c]	609[c]	6.2
Urine, mL/d, as is	3040[a]	2622[b]	3038[a]	2690[b]	6.3
Manure, g/d as is	3525[ab]	3276[b]	3616[a]	3299[b]	5.4
N, % digested	88.4	88.5	88.7	88.9	1.8
P, % digested	58.9	56.7	51.3	53.7	10.4
N					
Intake, g/d +	49.8	52.6	59.1	59.1	0
Feces, g/d	5.8	6.0	6.6	6.5	13.9
Urine, g/d	18.8[a]	9.8[b]	23.5[c]	14.4[d]	14.8
Total N excreted, g/d	24.6[a]	15.8[b]	30.1[c]	21.0[d]	9.4
Retained, g/d	25.2[a]	36.7[b]	28.9[c]	38.1[b]	6.7

* No Covariates used
+ Intakes calculated from analyzed dietary N and P values.
[a,b,c,d] Means with different superscripts differ by $P<0.05$.

Table 3. Nitrogen Excretion (Trial 2)*

Dietary CP	13.8% CP		16.1% CP		
Ractopamine, 20 ppm	-	+	-	+	CV
Water Intake, mL	5600	5600	5600	5600	--
Feed Intake, g/d	2100	2100	2100	2100	--
Feces, g/d, as is	473.5	434.4	465.6	458.9	11.9
Urine, mL/d, as is	3650.3	3661.7	3722.7	3592.9	5.6
Manure, g/d as is	4124	4096	4188	4052	5.1
N, % digested	88.8	90.8	90.9	90.5	1.4
P, % digested	55.4	57.5	55.6	58.2	9.5
N					
Intake, g/d +	47.56	50.19	56.39	56.38	--
Feces, g/d	5.49	4.59	5.17	5.24	12.1
Urine, g/d	14.6[b]	9.03[c]	18.0[a]	13.7[b]	9.8
Total N excreted, g/d	20.1[b]	13.6[c]	23.2[a]	18.9[b]	6.2
Retained, g/d	29.1[c]	36.1[a]	33.9[b]	36.2[a]	3.5

* No Covariates used
+ Intakes calculated from analyzed dietary N and P values.
[a,b,c,] Means with different superscripts differ by P<0.05.

Table 4. Total Slurry Ammonium Nitrogen (Trial 1)

		Ammonium N, ppm			
Dietary CP	Ractopamine, 20 ppm	d 0[1]	d 17	d 35	d 64
13.8%	-	5063[a]	4612[b]	4279[a]	3745[a]
16.1%	-	6144[b]	5976[a]	5091[b]	4933[b]
16.1%	+	4170[c]	4218[b]	3390[c]	3453[ac]
13.8%	+	2706[d]	3004[c]	2691[d]	2954[c]
CV		11.98	7.06	10.67	11.07

[abcd] Differing superscripts within a column indicate significance at P<0.05.
[1] Day of slurry incubation trial.

Table 5. Slurry Total Volatile Fatty Acid Content (Trial 1)

		Volatile Fatty Acids, mmol/L			
Dietary CP	Ractopamine, 20 ppm	D 0[1]	d 17	d 35	d 64
13.8%	-	53.32	105.52 [abc]	89.3 [ab]	82.26[ab]
16.1%	-	54.74	118.13 [a]	101.8 [a]	91.33[a]
16.1%	+	61.97	101.13 [bc]	79.1 [bc]	72.05[b]
13.8%	+	46.35	93.02 [c]	73.6 [c]	70.27 [b]
CV		8.26	12.44	12.30	16.08

[abc] Differing superscripts within a column indicate significance at P<0.05.
[1] Day of slurry incubation trial

Table 6. Odor Detection (DT) and Recognition (RT) Levels (Trial 1)

		d 0[1]		d 17		d 35		d 64	
Dietary CP	RAC, 20 ppm	DT	RT	DT	RT	DT	RT	DT	RT
13.8%	-	3697[a]	2041[a]	1874[a]	1060[a]	1869[a]	1156[a]	1005[a]	573[a]
16.1%	-	3753[a]	2036[a]	1115[b]	567[b]	2942[c]	1688[b]	2053[b]	1204[b]
16.1%	+	3574[a]	1975[a]	2052[a]	1224[a]	2727[ac]	1587[cb]	1076[a]	587[a]
13.8%	+	3213[a]	1675[a]	1923[a]	1139[a]	4235[b]	2480[c]	1055[a]	587[a]
CV		58.31	61.97	75.52	82.34	71.69	66.82	65.66	78.38

[abc] Differing superscripts within a column indicate significance at P<0.05.
DT=Detection Threshold, measure of when panelist correctly identified which air stream has a different odor from the other two air streams.
RT=Recognition Threshold, measure of when panelist can describe the odor.
[1] Day of slurry incubation trial

Table 7. Pit Composition

			Week 9 collection			
Week 9	PH	% DM	%Total N DMB	%Ammonia DMB	%Phosphorus DMB	%Potassium DMB
HCP[a]	7.6	1.43	20.8	15.9	4.34	6.93
RCP[b]	7.3	2.17	15.9	12.7	4.93	4.79
Significance P<	.008	.07	.025	.07	.02	.01
CV	2.6	49.3	25.7	33.9	15.4	25.7

[a] HCP = High crude protein diet
[b] RCP = Reduced crude protein diet

Table 8. Odor/Gases

	Week 9 collection				
	4 hr. Ammonia conc. (ppm)	Dilution Ratio (Fresh:Sample)	Ammonia (ppm)	Hydrogen Sulfide (ppm)	Blood Urea N (mg/dl)
HCP[a]	33.6	663	23.7	1.41	11.5
RCP[b]	17	472	11.3	.93	4.4
Significance P<	.026	NS	.023	.008	.0001
CV	40.2	42.4	24.1	21.1	30.9

[a] HCP = High crude protein diet
[b] RCP = Reduced crude protein diet

Table 9. Odor and gas analysis.

			Week 9 collection	
	4 hr ammonia conc. (ppm)		Dilution ratio (Fresh:Sample)	H_2S (ppm)
	Week 3	Week 9		
HCP[a]	13.6	21.3	533.2	.36
RCPF[b]	12.7	12.5	500.1	.25
Significance	NS	.03	NS	.02
CV	11.7	17.8	12.8	19.3

[a] HCP = High crude protein diet
[b] RCPF = Reduced crude protein diet with 10% soybean hulls.

Table 10. Effect of diet on ammonium nitrogen (NH_4-N), total nitrogen (TKN), dry matter (DM) and pH in fresh and stored manure.

Diet (% CP)*	pH	DM	NH_4-N	NH_4-N	TKN	TKN
		%	mg/L	%DM	g/L	%DM
Fresh manure						
Standard (15)	8.4[a]	13.1	5841[a]	4.9[a]	11.9[a]	10.4[a]
Syn. AA (11)	7.3[b]	17.2	5118[ab]	3.4[b]	10.4[b]	6.5[b]
AA+SBH (11)	6.8[b]	18.3	3892[b]	2.2[c]	9.3[b]	5.2[b]
AA+DSBP (11)	7.1[b]	17.1	4279[b]	2.6[bc]	9.9[b]	6.0[b]
SEM	.1	1.1	343	.3	.4	.6
Stored manure						
Standard (15)	8.4	4.7	4171[a]	8.7[a]	5.3[a]	11.2[a]
Syn. AA (11)	8.1	4.1	2623[b]	6.4[b]	3.2[b]	7.9[b]
AA+SBH (11)	7.9	4.8	2458[b]	5.3[b]	3.5[b]	7.6[b]
AA+DSBP (11)	7.9	5.0	1588[b]	3.1[c]	3.0[b]	6.0[b]
SEM	.2	.3	359	.6	.4	.6

* Diet I: Standard (Std) 15% CP; Diet II: 11% CP with synthetic amino acids (AA); Diet III: Diet II with 10% soybean hulls; Diet IV: Diet II with 10% dried sugar beet pulp.
[a,b] Different letter superscripts in a column is significant (P <0.05).

Table 11. Effect of diet on volatile fatty acid (VFA) in fresh and stored manure (mmol/L).

Diet (%CP)*	Ac	Pr	iBu	Bu	IV	V	Total
Fresh manure							
Std (15)	51	19	1.1	11	1.26	3.2	86
Syn. AA (11)	56	24	1.4	12	1.49	4.0	100
AA+SBH (11)	78	28	1.4	15	0.92	4.4	128
AA+DSBP (11)	65	23	1.5	12	1.41	2.3	105
SEM	7	3	.1	1.3	.2	.5	12
Stored manure							
Std. (15)	87	13	2.3	7	4.5	1.9	115
Syn. AA (11)	64	14	1.8	11	2.6	2.7	98
AA+SBH (11)	71	15	2.6	10	2.9	2.8	103
AA+DSBP (11)	49	8	2.1	4	2.5	1.1	67
SEM	7	2	.2	1	.3	.2	10

* Diet I: Standard (Std) 15% CP; Diet II: 11% CP with synthetic amino acids (AA); Diet III: Diet II with 10% soybean hulls; Diet IV: Diet II with 10% dried sugar beet pulp.
[a,b] Different letter superscripts in a column is significant (P <0.05).

Table 12. Effect of diet on room odor and gases at week 6

	Control	Soy Hulls	CV	Significance
4hr. Ammonia Conc. (ppm)	13.05	10.38	20.21	.017
Detection Threshold	2424.13	2162.5	38.58	NS
Ammonia (ppm)	4.00	3.63	23.36	NS
Hydrogen Sulfide (ppm)	1.03	0.701	20.99	.003

Table 13. Effect of diet on volatile fatty acids (VFA) in stored manure at week 6.

	VFA, mmol/L						
	Ac	Pr	IB	B	iV	V	Total
Control	57.7	16.7	2.1	16.8	2.0	1.7	97.0
Control+SH[a]	77.1	23.3	2.5	20.3	2.4	2.4	128.0
Significance *P*<	.0004	.0003	.013	.036	.043	.004	.0008
SEM	2.84	0.94	0.11	1.05	0.12	0.14	4.91

[a] Control Diet with 10% soy hull

Table 14. Effect of soyhulls in the diet on manure composition

	Control	Soy Hulls	CV	Significance
Manure Pit Volume (gal)				
Initial (as is)	840	840	--	--
Final (as is)	3209.6	2849.6	11.2	.10
Manure Drymater, %				
Initial	0.26	0.28	4.2	.07
Final	0.97	1.65	9.7	.0001
TN				
Initial (ppm)	510.2	438.5	9.7	.03
Final (ppm)	1681.3	2226.8	9.8	.001
Total pit accumulation, lb	41.0	49.7	11.5	.02
Ammonia				
Initial (ppm)	340.3	321.8	2.6	.006
Final (ppm)	1495.5	1817.2	7.3	.002
Total pit accumulation, lb	37.3	40.4	6.0	.05
Phosphorous				
Initial (ppm)	209.5	186.2	19.9	.33
Final (ppm)	500.7	606.5	5.8	.0005
Total pit accumulation, lb	11.9	12.6	15.1	.49
pH				
Initial	8.21	8.26	2.0	.69
Final	7.26	7.12	1.3	.03
Change	-0.93	-1.15	20.2	.19