

# Midwest Swine Nutrition Conference Proceedings



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Appreciation is expressed to the Indiana Farm Bureau and their staff for hosting the 2010 Midwest Swine Nutrition Conference and providing the facilities for this function.



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### Memorial

## Gilbert R. Hollis

November 26, 1939 to October 25, 2009

Gilbert R. Hollis, outstanding extension specialist and internationally renowned swine nutrition and production expert, died at the age of 69 in Champaign, Illinois at his son's home.

Gilbert was born in Poplar Bluff, Missouri, a twin with his brother Gregory, and the son of Orbra and Pauline (Cox) Hollis. He grew up on the family farm in Greenway, Arkansas where he attended the local high school. He attended the University of Arkansas where he received BS and MS degrees in animal sciences in 1961 and 1963, respectively. He earned his PhD in animal sciences from Purdue University in 1966. He married Willa Dean Johnson on August 27, 1961 in Greenway, Arkansas.

He served as professor and swine extension specialist at three prestigious institutions; the University of Florida (1966 to 1970), Texas A&M University (1970 to 1977), and ultimately, the University of Illinois (1977-2002). He was Professor Emeritus at Illinois from 2002 to 2009, where he continued to work in the Department of Animal Sciences teaching, mentoring graduate students, and organizing swine production science short courses.

Gilbert was the ultimate example of the swine extension specialist who many sought to copy, but few managed to emulate. Current in and completely familiar with the science of his discipline, he was also absolutely in tune with the problems and issues faced by his customers, the pork producers of Illinois. He was a great communicator both formally and informally and very adept at getting his message across to any audience. Who could ever forget that marvelous Arkansas accent booming across a producer meeting somewhere in the wilds of Illinois, extolling the audience to pay particular attention to a critical point.

Dr. Hollis was very much a "people person" who cared deeply about the industry he served and particularly the people in it. When they laughed, he laughed but when they suffered, he suffered with them and he would do anything and everything possible to try to help.

He developed a substantial and extremely successful international extension program. He was particularly active in Central America and the Caribbean where he was centrally involved in developing and presenting swine nutrition short courses as well as acting as a consultant for a number of organizations and individual producers.

Gilbert Hollis was an absolutely outstanding member of the faculty throughout his career and made a major contribution to the success of the Department of Animal Sciences, the College of ACES, and the University of Illinois. He was an outstanding colleague, always ready, willing, and able to help and advise under any circumstances, who mentored numerous undergraduate and graduate students and faculty.

Gilbert was an active member of the Midwest Swine Nutrition Conference. He served on the planning committee each year, was responsible for making leaflets, and circulating the program to the industry, extension personnel, and feed magazines to inform them of the conference.

# A Brief Review of the 10-Year History of the Midwest Swine Nutrition Conference

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The Midwest Swine Nutrition Conference (MSNC) is celebrating its 10<sup>th</sup> anniversary this year. The conference had its beginnings in 2001 with a small gathering at the Marriott Hotel on the east side of Indianapolis. The conference was held the afternoon before the opening of the annual meeting and trade show of the Indiana Pork Producers Association. The organizing group for the conference program consisted of two members from each of five universities – Purdue University, Michigan State University, The Ohio State University, University of Illinois, and University of Kentucky.

The five-state group of representatives from the five universities actually originated from an earlier group of nutritionists from three universities (Purdue, Michigan State, Ohio State) which was called the Tri-State Nutrition Group. This group was organized in the mid-1990s in order to interact and collaborate on swine nutrition research studies. In addition, they wrote a nutrition guide (Tri-States Swine Nutrition Guide) which was published in 1998 and was coordinated by Don Mahan (OSU). During several of their meetings, Dale Rozeboom (MSU) suggested

that the group give consideration to sponsoring a nutrition conference at some time in the future in one of the three states and the rest of the committee readily agreed.

In the spring of 2001, an invitation from the Indiana Pork Producers Association to develop an afternoon program that preceded their annual meeting and trade show was a natural incentive for the group to organize a nutrition conference. At about this time, Tip Cline suggested that the group be expanded, and representatives from the University of Illinois and the University of Kentucky were asked to join the group, forming the 5-state or “Midwest” group. The group developed a program for the first Midwest Swine Nutrition Conference. Tip agreed to chair the first conference and continued to serve in that role until this past year.

The first conference was a half-day event followed by a reception. It was a small group of about 30 people that consisted mostly of professors and graduate students from the five sponsoring universities and a handful of people from industry. The Indiana Pork Producers handled the registration

process and covered most of the cost of the conference. Five papers were presented and a proceedings book of the presentations was given to the attendees.

For the next three years, the MSNC was held at a local hotel in Indianapolis the day before the Indiana Pork Producers annual meeting and trade show. The conference was expanded to a full day and continued to slowly grow in the number of attendees. The number of feed company nutritionists and other industry personnel gradually increased as more people learned about the program.

In 2005, the MSNC was not able to schedule their annual conference in conjunction with the Indiana Pork Conference, so it was held at a different time. Also, it was becoming more difficult to find a large enough meeting room at a hotel that was not too costly. Brian Richert suggested to Tip Cline that the Indiana Farm Bureau might be willing to offer one of their conference rooms for the meeting. The president of Indiana Farm Bureau agreed to provide an expandable conference room at no cost, and the rest is history. The past two years, the MSNC has grown to the point that the entire conference room area was needed to handle the attendees, which numbered between 100 and 125.

Another change that occurred at about this time was the hiring of Cyndee Howell on a temporary basis to handle registration, serve as treasurer, develop a web site, and similar things of this nature. Cyndee lived in Indianapolis when she was hired and has since moved to Jefferson City, Missouri, but she still handles these activities for the group.

The first year, several potential sponsors were identified and funding was requested. Five organizations each made \$500 contributions. The next two years, six organizations sponsored the event. After the third year, Gary Cromwell offered to send personal letters to an expanded list of potential sponsors and did a more aggressive follow-up to those who did not respond to the initial invitation. That year, the sponsorship grew to 18 and has since grown to 40 sponsors this past year (Figure 1). Alltech and JBS United have been sponsors for all 10 years, and several others (Akey, Land O'Lakes/Purina, Elanco, Hubbard Feeds, PIC, Prince Agra Products, Zinpro) have been sponsors for all but one or two years.

In summary, the authors of this paper believe that the success of the Midwest Swine Nutrition Conference can be attributed to the following:

**Effective Planning** – The 5-state group of nutritionists travel to Indianapolis in January each year to plan the conference program. Close attention is paid to current topics of interest and to speakers that can best address those topics. A keynote speaker is identified to address some topic that is important to nutritionists and others in the swine and feed industries. As much as possible, representatives from the five sponsoring universities are included as speakers.

**Indiana Pork Producers Association** – During the early years, the Indiana Pork Producers Association covered most of the costs of the meeting and took care of the logistics. Without their help, we probably would have gone under.

**Indiana Farm Bureau** – The willingness of this organization to provide an excellent meeting room for the conference at no cost as well as provide space for a luncheon has been a key to the success of the conference.

**Sponsors** – Certainly this conference would not be what it is today without funding from the many sponsors who support the meeting with a gift of \$500. Without this source of revenue, it would not be possible to have a high quality program with excellent speakers.

**Topics and Speakers** – Numerous excellent presentations have been made by many speakers. Feedbacks from attendees indicate that our annual conference presents excellent subject material for their profession. A list of the subjects and speakers over the 10-year period is presented in Table 1. Subject areas and affiliation of speakers are shown in Tables 2 and 3.

**Proceedings** – An attractive and useful proceedings book has been published each year of the conference. Don Mahan coordinates the collection of papers from the speakers and the book of proceedings is published by The Ohio State University Press.

**Webcast** – Presentations have been recorded the past two years and webcasts were made available to expand the visibility of the conference and its sponsors into the Asian and other markets. The American Society of Animal Science has helped to fund this venture.

**Table I. Presentations at the Midwest Swine Nutrition Conference the past 10 years<sup>1</sup>**

<b>Year</b>	<b>Subject</b>	<b>Presenter</b>
2001	Organic Se for swine and human health Nutritional needs of pigs fed Paylean CLA and Omega-3 fatty acids Animal protein by-product ingredients Biotech food for livestock and humans	D. Mahan B. Richert M. Lindemann G. Pearl G. Hartnell
2002	Nutritional problems in the field encountered by a veterinarian Pig's need for vitamins E, A, and C High levels of B-vitamins Nutrition and pork quality HACCP regulations in the feed manufacturing business Proposed environmental regulations for CAFO Modifying diets to reduce odors Low-phytate corn and soybean meal on P excretion Feeding strategies to reduce nutrient excretion Mass balance	L. Rueff D. Mahan T. Cline M. Ellis W. Osburn R. Coffey A. Sutton G. Cromwell C. de Lange G. Hill
2003	Skeletal biology and sow longevity Conception enhancement Carcass modifiers Automatic sorting technologies for market hogs Antibiotic withdrawal and resistance Terminal use of antimicrobials – the Danish experience Viable antibiotic alternatives Practical problems if antibiotics are banned Feeding sows in a group-housing environment	M. Orth D. Levis S. Radcliffe S. Moeller M. Newman J. Pettigrew M. Lindemann B. Straw D. Levis
2004	Feeding the modern sow Energy systems - NE vs. ME Acidification of starter diets High quality protein sources for young pigs Feeding considerations for wean-to-finish systems Adipocytes and energy regulation Nutrition and health of swine Identification of limiting amino acids in diets to minimize N excretion Phytase in diets and P movement in soils Nutrition and air quality	D. Mahan J. Patience S. Radcliffe T. Cline B. Wolter M. Spurlock R. Johnson G. Cromwell B. Joern A. Sutton
2005	Nutrition concerns as viewed by the feed industry Nutrition concerns as viewed by a swine veterinarian Current issues of importance for feed manufacturers Amino acid nutrition of swine Modeling Ca and P requirements for growing-finishing pigs Host and intestinal microbiota Dietary effects on gastro-intestinal microbial populations Dietary fiber for sows Organic and inorganic Cu, Zn, Fe, and Mn Organic Se fed to swine - potential effects on human health	J. Kelley K. Lehe S. Traylor D. Baker A. Pettey R. Gaskins J. Pettigrew J. Crenshaw G. Hill D. Mahan

2006	World pig production – opportunity or threat Implications of nutrient-gene interactions Mineral status of high producing sows Corn and soybeans - what's here and what's coming Biosecurity - current and emerging threats to animal production Nutrition and management on hemorrhagic bowel syndrome DDGS production – present and future DDGS - nutrient content and digestibility	D. Orr S. Radcliffe D. Mahan D. Jones R. Norton W. Hollis M. Gibson H. Stein
2007	Animal sciences in academia Managing grouped sows Antioxidants and swine mortalities Porcine circovirus Ingredient and hog prices in the coming months Energy sources - how do we cope? Feeding DDGS to pigs - what's new? Dietary fat and carcass quality	R. Easter J. Salak-Johnson D. Mahan T. Gillespie R. Plain M. Lindemann H. Stein M. Latour
2008	Nutritional issues facing the swine industry DDGS in swine diets Tools to cope with current economics NRCS nutrition and management standards that affect pig feeding Segregated parity structure in sow farms Mineral composition of current pigs and pigs of the past Energy from protein and fiber - a case for NE systems Novel soybean products for swine	B. Borg G. Hill R. Coffey A. Sutton D. Boyd D. Mahan C. de Lange H. Stein
2009	Global demand for animal protein - implications for the feed industry DDGS and air emissions High levels of DDGS for swine Rice and barley - do they improve health in nursery pigs? Feeding gilts and sows Mannan oligosaccharides (MOS) for sows and weanling pigs Natural and synthetic vitamin E - new discoveries CLA in grow-finish and lactation diets	D. Armstrong W. Powers G. Cromwell J. Pettigrew L. Greiner M. Lindemann D. Mahan B. Richert
2010	10-year history of MSNC Perspectives from Washington - policies impacting animal agriculture Environmental-monitoring research at Purdue NIR of feedstuffs and enhancement for predicting nutrient availability Salmonella transmission, dissemination, colonization, and control Vomitoxin - how do we correct the problem? Current status of NE Mammary gland metabolism and amino acid nutrition A perspective on changes in the feed industry the next 10 years	T. Cline and G. Cromwell L. Randel B. Richert and S. Radcliffe J. Black P. Ebner D. Mahan J. Pettigrew N. Trottier P. Lyons

<sup>1</sup>Based on the proceedings of the Midwest Swine Nutrition Conference.



**Table 2. Subject areas presented at the Midwest Swine Nutrition Conference from 2001 to 2010**

<b>Subject area</b>	<b>Number of presentations<sup>1</sup></b>
Environmental issues (general, nutrient excretion, odor, etc.)	10
Feed additives (antibiotics, microbial supplements, other)	10
Sow nutrition and management	10
Global, national, and university perspectives	8
Mineral nutrition	8
Nutritional issues of concern, regulations	7
DDGS	6
Swine health and diseases	6
Energy nutrition and energy systems	5
Feedstuffs (conventional and alternatives)	5
Carcass modification with nutrition and additives	5
Protein and amino acid nutrition	4
Fat and fatty acid nutrition	3
Genetically modified feedstuffs	3
Gut health of pigs	3
Vitamin nutrition	2
Economics	2
Other	5

<sup>1</sup>Some of the presentations are included in more than one subject area.

**Table 3. Speakers at the Midwest Swine Nutrition Conference from 2001 to 2010**

<b>Affiliation</b>	<b>Committee members</b>	<b>Non-committee members</b>	<b>Total number of speakers</b>
<b>Sponsoring universities</b>			
Purdue University	8	7	15
University of Illinois	7	6	13
The Ohio State University	11	1	12
University of Kentucky	8	4	12
Michigan State University	3	5	8
Other universities			7
<b>Industry</b>			
Feed industry			9
Swine industry			4
Veterinarians			4
Government relations			1

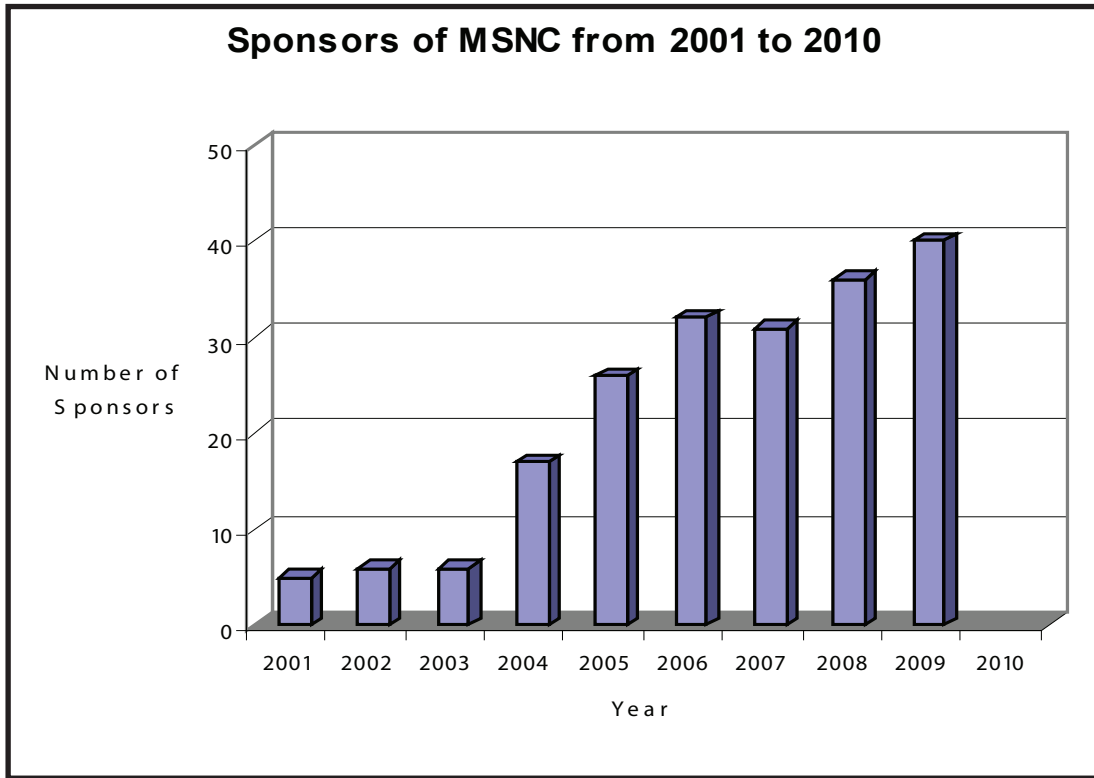


Figure 1. Number of sponsors of the Midwest Swine Nutrition Conference from 2001 to 2010. The number of sponsors for 2010 was not available at the time of the press deadline, but the number of 2010 sponsors can be found at the beginning of this proceedings.

# Emerging Policy Issues Impacting Animal Agriculture

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## Summary

Animal agriculture is at the center of several emerging policy issues that present challenges to the future of food animal production in the United States. Driving some of these policies is the pervasive negative media attention regarding conventional production practices. Confined animal feeding operations are seen as problematic for animal welfare, especially the use of gestation crates, veal crates and battery cages, causing state governments and the federal government to consider restrictions on these practices. Animal agriculture is also implicated as a source of concern in the area of antibiotic resistance. Congress and federal agencies are proposing to curtail the use of antibiotics in food animal production, with a primary focus on discontinuing use for growth promotion.

Climate change is another area where animal agriculture is coming under fire, with some sources claiming that a major percentage of all greenhouse gas emissions come from food animal production. As a result, policies are being considered to control emissions, which could drive up production costs. Amidst all of these challenges is the need for an increased food supply to meet an increasing global demand for food. Food security could present one of the greatest opportunities for animal agriculture to promote its value and contribution to society.

With all of these challenges and opportunities comes the need for additional investment in the animal sciences. Recent changes to research programs at the U.S. Department of Agriculture (USDA) are reason for both hope and concern. Funding for USDA's Agriculture and Food Research Initiative is poised to receive important increases, but structural changes within the program have raised concerns that the animal sciences may not see the full benefit of increased funding for the program. All of these factors reinforce the need for animal scientists to be engaged in the public policy making process to ensure that the voice of animal agriculture is not lost as policy makers consider the fate of food animal production in the United States.

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## Introduction

Animal agriculture and the animal sciences are facing a number of significant challenges shaping the future of food animal production in the United States. These challenges range from negative television reports to policy proposals regarding antibiotic use and animal welfare that lack a balanced scientific basis. This paper will discuss the current status of emerging policy issues and how they may impact the future of animal agriculture.

### **Public Perception of Animal Agriculture**

One of the driving forces creating challenges for animal agriculture is negative media shaping

public perception. When the general public hears about animal agriculture in a newspaper or on the television, the message is almost always negative. For example, two of the major news networks, ABC and CBS have run negative stories about production practices for food animals. On January 26, 2010, ABC News ran a story criticizing the use of tail docking in dairy cattle. On February 9, 2010, CBS News presented a story regarding the use of antibiotics in food animal production, linking their use to human antimicrobial resistance. Both of these stories reached a broad cross-section of the nation with a message that current production practices are causing problems. Unfortunately, the scientific basis for these stories was relatively thin and lacked a balance of multiple perspectives.

Even Comedy Central is getting in on the act, with Steven Colbert of The Colbert Report interviewing Jonathon Safran Foer, the author of “Eating Animals”. Despite the comedic bend of the story, the anti-meat agenda came through loud and clear, reaching an audience that largely does not have an independent base of knowledge about agriculture.

The Humane Society of the United States (HSUS) is a major factor in driving the public perception about animal agriculture. HSUS has a significant media budget and has been effective in advancing its agenda against animal agriculture through print media and television ads. There has largely been a gap in providing animal agriculture’s perspective on these issues and the industry has remained on the defensive. On a positive note, in 2010, the Center for Consumer Freedom launched Humane Watch to counter some of the anti-animal agriculture efforts of HSUS and provide more balance to the battle for public perception.

The pervasive nature of negative media towards animal agriculture is not only impacting public perception, it is also driving public policy debates. This is particularly in the areas of animal welfare and antibiotics use.

## **Animal Welfare**

The issue of animal welfare is perhaps one of the most difficult for production agriculture. Images of abused animals leave deep imprints in the psyche of the public and create the perception that those engaged in food animal production routinely neglect to provide an appropriate standard of care for their animals. It is also becoming increasingly difficult to explain the rationale for using confined animal feeding practices such as gestation crates and battery cages. This has driven heated policy discussions at the state, federal, and international levels.

Much of the action on animal welfare policy has originated at the state level, led by efforts of HSUS to restrict confined animal feeding operations. As with many social policy issues the State of California has been a leading force for change. In 2008, California voters passed a ballot initiative that prohibits the confinement of animals in such a way that they are not able to turn around freely, lie down, stand up, and fully extend their limbs. The measure passed with an overwhelming 63 percent support. While other states have voted to restrict the use of gestation

and veal crates, this was the first time voters had also acted to prohibit the use of battery cages in poultry production.

Ohio has been another major battleground in animal welfare policy. In 2009, the animal agriculture industry advanced a constitutional amendment to create a 13-member Ohio Livestock Care Standards Board for the purpose of establishing standards governing the care of livestock and poultry. The measure received support from over 63 percent of Ohio voters and was seen as a victory for production agriculture and a defeat for HSUS. HSUS mounted a campaign for a ballot initiative in 2010 that would have mandated restrictions on battery cages and gestation crates, apart from the activities of the Board created by the 2009 constitutional amendment. In an eleventh hour attempt to avoid another contentious ballot fight, Ohio Governor Ted Strickland brought the Ohio Farm Bureau and HSUS together to forge a compromise. His efforts were successful, and an agreement was announced on June 30, 2010. The compromise would phase out the use of battery cages, gestation crates and veal crates, as well as prohibiting the transportation of “downer” animals to market and requiring humane euthanasia for sick or injured animals. Both sides view the agreement as positive development to improve animal welfare for the future.

Legislation has also been introduced in the United States Congress to address animal welfare issues. On March 2, 2010, Representative Diane Watson introduced the Prevention of Farm Animal Cruelty Act. The legislation would prohibit federal agencies from purchasing food animal products unless the animals were raised in a way that enabled them to stand up, lie down, turn around freely, and fully extend all limbs. The bill has been referred to the House Agriculture Committee, but has not seen any action since its referral.

The response from the private sector is perhaps the most important development in the area of animal welfare. Major corporations such as Wal-Mart, McDonalds, Wendy’s and Subway have taken steps to ensure that they are procuring food animal products from producers employing certain production practices. Cage free eggs have been the primary driver for change in the corporate world, but there are signs that pork producers will be moving away from gestation crates, as well. Smithfield Foods, has been exploring the transition away from

gestation crates, although have recently backed away from their initial timeline to move completely away from their use. In the end, demands of the free market may prove to have more impact on production practices than state or federal government initiatives.

## **Antibiotic Use in Animal Agriculture**

The issue of anti-microbial resistance has been debated for decades, but has come under increased attention in recent years. International governments, particularly in Europe, have already taken action to restrict the use of antibiotics in animal agriculture. Now, the U.S. Congress is debating policies surrounding antibiotic use in animals and the Food and Drug Administration (FDA) has recently released guidance on the “judicious” use of antibiotics.

The Preservation of Antibiotics for Medical Treatment Act (PAMTA) is legislation currently being considered by Congress to restrict the “non-therapeutic” use of antibiotics in animals. The bill would phase out the “non-therapeutic” use of any kind of penicillin, tetracycline, macrolide, lincosamide, streptogramin, aminoglycoside, or sulfonamide; or any other drug or derivative of a drug that is used in humans or intended for use in humans to treat or prevent disease or infection caused by microorganisms. The term “non-therapeutic use” is defined in the legislation as the use of critical antimicrobial animal drug as a feed or water additive for an animal in the absence of any clinical sign of disease in the animal for growth promotion, feed efficiency, weight gain, routine disease prevention, or other routine purpose. Numerous hearings have taken place regarding PAMTA legislation, along with briefings conducted by both “pro” and “anti” PAMTA groups to examine the issue of antibiotic resistance.

Given the current political climate in Washington, D.C., legislative action on controversial issues such as PAMTA is less likely. As a result, the FDA is looking to shape antibiotics policy through administrative actions. On June 28, 2010, the FDA today rolled out proposed guidance on “The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals”. The guidance is “intended to help reduce the development of resistance to medically important antimicrobial drugs used in food-producing animals.” The guidance recommends the implementation of policies that would limit the use of medically important antimicrobial drugs in food-producing animals to

situations where the antibiotics are necessary for assuring animal health. The guidance also stresses the need for veterinary oversight. FDA asserts that these measures would reduce overall use of medically important antibiotics and decrease antimicrobial resistance.

A major distinction between the FDA guidance and the PAMTA legislation is the view on using antibiotics for disease prevention. PAMTA would restrict antibiotic use to only the treatment of sick animals. The FDA guidance acknowledges the value of preserving the use of antibiotics for both prevention and treatment. In both cases, the use of antibiotics for growth promotion would be phased out.

The FDA guidance was met with support from groups such as the Union of Concerned Scientists and the Pew Foundation, who have been pushing for restrictions on antibiotic use, including the enactment of PAMTA. While they support the FDA guidance, they would also like the FDA to have gone farther in its restrictions.

The animal industry was less positive about the FDA action. The National Pork Producers Council (NPPC) criticized the FDA guidance as lacking a strong scientific foundation. They argue that the definitive connection between the use of antibiotics in food animals and human antimicrobial resistance has not been made. The Animal Health Institute (AHI), a group representing the animal pharmaceutical industry, was more measured in its response, focusing on the need for stakeholder involvement in the regulatory process and the importance of maintaining availability of drugs to ensure animal health.

## **Climate Change Policy**

Climate change has been a major topic of debate in the United States and across the globe in recent years. A major international climate change conference took place in Copenhagen in December where governments from across the world met to discuss policies to mitigate greenhouse gas emissions. Controversy has swirled about the science behind climate change and “climate gate” has created a number of questions about scientific foundation surrounding global warming.

Couched within this debate is the impact animal agriculture has on climate change. The Food and Agriculture Organization (FAO) estimated in 2006 that animal agriculture contributed 18 percent of

all greenhouse gas emissions. This analysis has come into question as scientists from the University of California at Davis have challenged the FAO's calculations, claiming that FAO did not appropriately factor transportation across all industries. They assert that food animal production actually accounts for only about three percent of all greenhouse gas emissions in the U.S.

Addressing climate change is a high priority for President Obama and a number of Congressional leaders. In summer 2009, the U.S. House of Representatives passed comprehensive climate legislation. The U.S. Senate has considered a number of different climate change proposals, but has not been able to move legislation through the process. Given the controversial nature of climate legislation and the impending November mid-term elections, it appears unlikely that the issue will be resolved in the near future.

In the meantime, the Environmental Protection Agency (EPA) has taken initial steps to regulate greenhouse gases. In December 2009, EPA issued a finding that greenhouse gases threaten public health and the environment. The EPA's finding of endangerment opens the door for EPA to regulate the emission of greenhouse gases through the Clean Air Act authorities. The gases covered by the finding are: carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride (SF<sub>6</sub>). This finding has drawn considerable opposition and a number of lawsuits have been filed to challenge the validity of EPA's finding.

Should regulations or legislation place restrictions on greenhouse gas emissions, animal agriculture could be negatively impacted. Energy and feed prices would likely rise due to the policy changes, placing additional pressures on producers. In addition, new regulations could place restrictions on production practices and waste management.

## **Trade and Animal Agriculture**

In an increasingly global economy, trade policy is critically important to animal agriculture in the United States. The USDA Foreign Agriculture Service estimates that U.S. agriculture exports in 2010 will reach approximately \$98 billion. Of that, approximately \$20 billion will come from livestock,

dairy and poultry exports. Agricultural imports are estimated to be \$77 billion, meaning that agriculture will continue to provide a positive contribution to the U.S. balance of trade.

However, the recent global recession has resulted in many countries instituting protectionist measures to support domestic industries. An example of these barriers came during the 2009 outbreak of H1N1, also known as "swine flu". Countries like Russia and China, major markets for U.S. producers, restricted exports from the U.S. under the guise that pork from the U.S. was not safe because of the prevalence of H1N1 in the country. These restrictions were counter to established science showing that H1N1 does not pose a food safety risk.

While these markets eventually reopened, even the temporary halt to trade caused economic pressures to the U.S. industry. Given protectionist tendencies of some major trading partners, the U.S. industry, and the U.S. government will need to remain vigilant to ensure that trade policies are based in science.

## **Food Security**

While animal agriculture is facing some serious policy challenges in the areas of animal welfare and antibiotics use, the issue of food security represents one of the best opportunities to advance the industry. According to the Food and Agriculture Organization (FAO), global population is predicted to grow by 2.4 billion over the next forty years. FAO further estimates that food production will need to double in forty years to accommodate the rise in world population.

In order to double food production, FAO has asserted that twenty percent of the increased production can come from adding new land into production, ten percent from increased production intensity, and seventy percent from adoption of new and existing technologies. Of this increase in world food needs, demand for animal protein will also continue to grow at a rapid pace, particularly in developing countries where economies are growing and greater populations will be able to afford animal products. This means that advances in agricultural science will be even more critical over the coming decades and provides a strong rationale for increased investments in the animal sciences.

## Investment in Animal Science Research

The 2008 Farm Bill included some significant changes to USDA's extramural research agency. The Cooperate State Research, Education and Extension Service (CSREES) was transformed into the National Institute for Food and Agriculture (NIFA). The change was driven by the stagnant nature of funding for USDA research and a perception that other federal research programs were of higher quality. Policy makers intended the new agency to operate more like the National Science Foundation and the National Institutes of Health, in hopes of reinvigorating support for USDA's science programs. NIFA would be headed by a Presidentially appointed Director. Dr. Roger Beachy was appointed by President Obama and he has been on the job since October 2009.

The 2008 Farm Bill also included provisions to transform USDA's premier competitive grants program, the National Research Initiative (NRI) into the Agriculture and Food Research Initiative (AFRI). Again, this was designed to energize support for USDA's extramural research programs. And, if early funding trends are any indication, these changes may have had some impact. In fiscal year 2010, the AFRI program received \$262 million. For fiscal year 2011, President Obama has requested \$429 million for AFRI. On June 30, 2010, the House Agriculture Appropriation Subcommittee marked-up its version of the 2011 Agriculture Appropriations Bill and included \$312 million for the AFRI program. While the House number is significantly lower than the President's request, it still reflects an almost 20 percent increase for the program.

The added attention and investment in the AFRI program is a welcome step for the overall advancement of agricultural science. However, NIFA's implementation of the AFRI program has raised some serious concerns in the animal science community. For example, under the 2010 AFRI RFAs, funding targeted at farm animal research under the Foundational RFA is only 8.6% (\$22.5M) of AFRI's \$262M budget and 5.6% of the total competitive grants budget at NIFA. It is important to recognize that some additional investments in animal science may come from the challenge area RFAs. For 2010, the Food Safety, Climate Change and Food Security RFAs do provide for some investment in animal related research. However, because the

challenge areas topics are narrowly focused and will change from year to year, it is critical that the opportunity for progress in solving issues related to food animals be maintained.

In addition to the narrow focus of the challenge area topics; there is concern about the use of forward funding for the challenge area RFAs, which means that multi-year grants are funded on a year-to-year basis relying on continued Congressional appropriations. This funding strategy is similar to how the National Institutes of Health fund some of their programs, but opens the door to potential unintended consequences. Future RFAs may not be viable if sufficient funds are not provided for the program. This means that a sustained effort to grow the program will be needed to ensure that AFRI is capable of producing robust RFAs on an annual basis.

President Obama's budget request for the AFRI program in fiscal year 2011 is \$429 million, which represents a significant increase over the \$262 million appropriated for the program in 2010. Should Congress provide sustained increases at the rate proposed by the Obama Administration, forward funding will be a more viable scheme. However, the historical funding trends for USDA competitive grants programs, coupled with current budgetary pressures makes annual increases of this magnitude less likely.

The House Agriculture Appropriations Subcommittee marked-up its version of the 2011 Agriculture Appropriations Bill in June 2010 and provided \$312 million for the AFRI program. While this represents a significant percentage increase, it would only allow for approximately \$50 million in new challenge area RFAs in 2011. For disciplines such as the animal sciences who felt that insufficient opportunities were presented by the 2010 RFAs, having such a significantly smaller set of RFAs in 2011 would be problematic.

Another potential unintended consequence of the new system is the negative impact on single investigator and new investigator driven science. While there is clearly value in supporting larger, longer and more integrated grants, a balance needs to be maintained to ensure that creative investigator driven research is not discouraged or lost. The Foundational RFA can help play a role in supporting creative investigator driven research and it is critical that funding for this RFA be maintained, and increased, if possible. At most research institutions, expectations for promotion and tenure are related to

independent research awards for faculty members and these are the least likely individuals to be able to head a multi-PI proposal effectively. Balance is needed which includes award opportunities to promote the best researchers forward to productive careers of research contribution. Multi-institutional grants also have implications on indirect cost recovery. In the case of a multi-institutional grant, there is a risk that indirect costs will take up a disproportionate amount of the award if each participating institution takes a percentage, plus an additional amount for the lead institution.

As the AFRI program continues its transition, and future RFAs are developed, it is important to remember the value of farm animal research to the future of animal agriculture in the U.S. AFRI is the primary competitive grants program supporting basic

and applied research in farm animals. Consequently, the portion of the NIFA-AFRI budget dedicated to farm animal research should reflect the critical importance of animal agriculture to the overall agriculture economy.

Continued erosion of AFRI funding dedicated to food animal research will continue to stymie recruitment of top notch young investigators to conduct cutting-edge science relevant to animal agriculture. This pipeline is necessary to train the next generation of scientists and industry leaders dedicated to research in animal agriculture. A vibrant competitive grants program dedicated to farm animal research is fundamental to sustaining the abundance of a high quality, safe, and affordable supply of meat, milk, fish and eggs produced in the U.S. This will greatly benefit consumer and help foster the continued success of animal agriculture.



# Evaluation of three commercial mycotoxin inhibitors added to Vomitoxin (DON) contaminated corn diets for weanling pigs: A Report from the NCCC-042, S-1044, and NCERA-89 Regional Committees on Swine Nutrition and Management<sup>1</sup>

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## Summary

A regional study involving 12 experiment stations using a total of 904 weanling pigs in 27 replicates evaluated three commercial mycotoxin inhibitors added to two different vomitoxin (DON) contaminated corn sources. The first corn analyzed 2.0 ppm DON while the second analyzed 7.0 ppm DON. The complete diet, mixed and provided in meal form from one mixing facility, was calculated to contain 1.0 and 3.9 ppm DON, respectively. The companies that produced these mycotoxin inhibitors were asked to recommend their level of product (Defusion®, Integral®, Biofix®) to be added to the diets. The study was blinded from participating companies and investigators to prevent bias. The test period was conducted after a 10 day adjustment period to a common diet. The test period that evaluated these mycotoxin inhibitors was conducted from 10 to 31 day post weaning. The results showed that the high DON corn diet reduced performance responses more severely than diets with low DON contamination. Defusion, added at 10 lb per ton was the most effective mycotoxin inhibitor in our study in both corn sources while the other mycotoxin inhibitors were ineffective. Lighter weight pigs were more severely affected by the DON contaminated diets than pigs of a heavier body weight, but both weight groups responded positively to Defusion. It is questionable if the feeding of a low DON contaminated corn would justify the added expense of the product while it was beneficial when DON was at a high level.

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## Introduction

At the regional swine nutrition meeting in January 2010, the North Central Coordinating Committee on Swine Nutrition (NCCC-042) recognized the extensive vomitoxin (DON) contamination present in much of the 2009 corn crop in the United States. The contamination was also found to be high in corn by-products such as

Dried distillers grains with solubles. The problem was presented to other regional committees (S-1044 and NCERA-89) who had similar concerns. A combination of investigators from these three groups evaluated how our committees could help the swine producer overcome the DON problem and how to best continue feeding this year's corn crop, particularly since there were no proven mycotoxin inhibitors on the market. It was reported that many pigs completely refused to eat diets containing these DON contaminated corn sources which ultimately

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<sup>1</sup>Appreciation is expressed to OARDC Feed Mill Manager Jack Bardall and his crew for procuring the corns, mixing, bagging, wrapping, and transporting the complete diets to the designated stations.

could have serious implications on animal health, welfare issues, and economic returns for the swine producer.

Fortunately most of the DON contaminated corn in the U.S was not at a level that seemed to affect cattle or poultry while swine appeared to be the most sensitive to the mycotoxin. Unfortunately, there were no FDA approved mycotoxin inhibitor products available, but there were some products on the market that were reported to be of benefit. However, they were not studied or reported in the literature within the public domain. It was decided to conduct a joint regional project to evaluate three of the major products available, and to share the results with the farm and feed community as quickly as possible. The goal was to evaluate the mycotoxin inhibitor products as to their effectiveness, and how we would recommend feeding the remainder of this year's corn crop. Our desire was to not only complete the study rapidly but also to report the results widely in lay publications for potential use by the swine and feed industry. There were 12 stations that could conduct the study in a timely manner and they and the principal investigators are identified in Table 1.

## Procedures followed

Corn from three sources was purchased with different DON levels for conducting the project. The first source was the cleanest source of corn (DON = 1.9 ppm) available. This corn source was fed during the pretrial period for an approximate 10 day period in order to allow the weanling pigs to get started on a common diet (without any mycotoxin inhibitor added) and to overcome the normal post weaning lag in growth and feed intake. The other two corn sources used in the subsequent test diets analyzed 2.0 ppm or 7.0 ppm DON, the former source analyzing somewhat lower than expected. A complete profile of other major mycotoxins analyzed in these corn sources by HPLC determined that DON was the major mycotoxin present (Table 2), that the other mycotoxins, particularly T-2 Toxin and zearalenone were present but at levels below that which would cause problems.

The pretest diet was fed for approximately 10 days and was comprised of dietary feedstuffs normally fed in a phase 1 diet to weanling pigs. Test diets during the following 21 day test period were formulated to utilize as much corn in the diets as possible in order to best test the efficacy of the three

selected mycotoxin inhibitor products. Only one diet was fed from the 10 to 31 day period for each treatment group. The companies were contacted and they all agreed to have their products evaluated.

All cooperating stations fed the same pretest diet, used the same corn sources, and used the same diet mixtures (including the pretest diet), mixed at one location (OARDC feed mill, Wooster, OH) and transported to each cooperating station in early February 2010. All diets were formulated to meet or exceed current NRC (1998) swine nutrient requirements (Table 2). Although the products were mixed in some cases a few weeks prior to being fed, most of the studies were done shortly after the diets arrived at the various stations (see Table 1 for starting dates). The three products to be incorporated into the test diets (Defusion<sup>®</sup>, Integral<sup>®</sup>, and Biofix Plus<sup>®</sup>, was added at the expense of corn starch to maintain the same nutrient profile of the remaining dietary constituents. The three commercial mycotoxin inhibitor products were purchased on the open market to ensure that the companies would not be accused of preparing special products for this trial. Each contributing company was given the opportunity to evaluate the corn mycotoxin assay results, the diet formulas that the products were to be added, and to recommend the incorporation level of their product into the test diets with the two corn sources. The amount of products added to the 1.0 ppm diets were (Defusion 10 lb/ton; Integral 4 lb /ton and Biofix Plus 8 lb/ ton), while the amount suggested for the 3.9 ppm diets were (Defusion 10 lb/ton; Integral 6 lb/ton; and Biofix Plus 8 lb/ton). In addition, the treatment and product identification was blinded not only to the company but also to the investigators. Each investigator was asked to collect performance data but to also evaluate other signs, denoting the date and reasons why pigs might be removed from the study. At the completion of the study, each company and investigator was again given the opportunity to review the final results without knowing which treatment represented specific products. All of this was done to ensure that bias would not enter into the conduct of the trial or data interpretation.

The three products evaluated were from the following organizations: BioMin (Biofix Plus<sup>®</sup>), Akey (Defusion<sup>®</sup>), and Alltech (Integral<sup>®</sup>). Vomitoxin consumption has been reported to result in reduced feed intakes, reduce body weight gains, and sub-clinical immune suppression. High levels of vomitoxin may produce intestinal lesions, vomiting,

and complete feed refusal. Pig gain and feed intake performance criteria were the measurement traits evaluated in this study. A short explanation of the products and how each product might function in reducing the effects of DON follows:

**Biofix Plus** (Bio Min) contains yeast cell wall, natural microbials, and diatomaceous earth (clay) which may be effective in reducing DON and other mycotoxins.

**Defusion** (Akey) is a blend of preservatives, antioxidants, amino acids, and direct-fed microbials which is thought to decrease some of the toxic effects of vomitoxin in pigs.

**Integral** (Alltech) is a yeast cell wall that has been modified and may serve as an adsorbent of dietary mycotoxins.

The completed trial data was statistically analyzed using conventional SAS analysis of variance procedures. Although pigs were allotted on initial body weight at weaning they were fed a common diet for an approximate 10 day period. Consequently, the weights at the beginning of the test period differed slightly. Thus the 10 day weights were adjusted by covariate analysis (to use a common initial weight within replicate from 10 to 31 day) to ensure that the responses were not affected by differences in weight at the beginning of the test period.

## Results

The complete set of data from all stations involving all replicates is reported in Table 3. There were 12 stations that conducted the trial involving a total of 904 pigs. Some replicates contained pigs of an initially lighter or heavier weight at weaning. Therefore six of the lighter weaning weight and seven replicates of the heaviest weight were analyzed independently to see if there were different initial weight responses to the DON contaminated corn sources and the various mycotoxin inhibitors. The performance responses from the 27 replicates are reported in Table 3 while the effect of light or heavy weaning weight pigs are presented in Tables 4 and 5, respectively.

The pretest diet fed for an approximate 10 day period resulted in good performance responses, but two pigs were removed before the product evaluation test period started. Their removal was due to unthriftiness and loss of body weight. In general, the pretest diet that contained a low innate level of DON

(0.80 ppm) did not appear to affect pig gains or feed intakes (Table 3).

Feeding the treatment test diets (days 10 to 31 post weaning) clearly resulted in different performance responses to the two different corn sources. Pigs consuming the 7.0 ppm DON corn (diet calculated at 3.9 ppm DON) had reduced pig body weight gains and feed intakes each week of the test period compared to the corn that tested 2.0 ppm DON (diet calculated 1.0 ppm DON). Unfortunately we did not have access to corn without DON contamination and could not make a comparison to such corn. There was no incidence of feed refusal for either of the two test corn sources, but feed intake was reduced when the higher DON contaminated corn was fed. There were a total of five pigs removed from the study. Although unthriftiness of pigs was generally recognized throughout the study it was not severe enough to remove pigs from the trial. Of those pigs removed, the prevailing observation was a decline in body weight, limb immobility, and pneumonia. There was evidence of swollen vulvas when pigs consumed the 3.9 ppm DON diet but this was probably reflective of zearalenone contamination not DON. There was no reported incidence or evidence of intestinal hemorrhages which would be indicative of T-2 Toxin. As expected, the major negative response from DON contamination appeared to result in reduced gain, reduced feed intake, and a general unthriftiness, the latter response was most likely because of the low feed intake.

Comparison of the three commercial mycotoxin inhibitor products for all stations for the 27 replicates is reported in Table 3. For the low Don contaminated corn only Defusion proved to be effective by increasing pig gains and feed intakes during week 1 and 3 of the test period over that of the negative control diet. The effect of the other mycotoxin inhibitors to the diets was statistically similar to the negative control. The overall growth rate and feed intake did not, however, differ significantly for most of the trial for two of the three mycotoxin inhibitors products, but there was an apparent numerical advantage to Defusion. Although this level of DON is reported to be tolerated by the young pig, our results would indicate that its additional expense to diet cost may not be cost effective when a low level ( $\leq 1$  ppm) of DON is fed to weaning pigs.

In contrast, when the high DON corn diets (calculated at 3.9 ppm DON) were fed those pigs consuming the diet with Defusion weighed more at the end of the trial, gained more weight and

consumed more feed during each week of the trial than those fed the control or Integral or Biofix Plus mycotoxin inhibitors.

When pigs were evaluated by weaning weight groups they responded to the two corn sources and mycotoxin inhibitor products somewhat differently. The results of the lighter weight pig group (Table 4) indicated that their response to the DON contaminated corn source was more pronounced than the heavier pig group (Table 5). In the light weight group there was a clear benefit to Defusion for both DON contaminated corn sources, whereas there was no response to the other two products. The benefits of Defusion were evident during the initial week of the test period and continued throughout the remainder of the trial. In the heavier pig group the same general trends occurred but the results were not as dramatic as when the lower DON contaminated corn source was fed. Again with the higher DON contaminated corn, Defusion still proved to be the superior mycotoxin inhibitor in both growth rate and feed intake during each week of the trial.

## Discussion

Although Defusion was superior in our trial, the corn used in these treatment diets was primarily contaminated with DON and not the other *Fusarium* molds. How the other mycotoxin inhibitor products used in our study would respond with corn that also contained zearalenone, T-2 Toxin or aflatoxin is unknown. It is unusual that corn mycotoxins are predominated by a single mycotoxin and in some cases the other products might be effective against the other mycotoxins.

Because Defusion was also added at a high level, it is not known what a lower dietary inclusion level would produce.

There are several lessons and recommendations that we can make from this study.

It is important to analyze for the various mycotoxins present in corn sources or their by-products when fed to swine. The “quick test” done by most elevators is a good starting point for determining the amount of contamination but these tests are not completely reliable and highly variable. Once a large quantity of corn is stored it is a good idea to test the entire bin (several probes) and be analyzed by a recognized laboratory using modern techniques. Be sure to test at various sites in the

bin so as not to isolate a “hot spot”. Mycotoxin contaminated grains seem to accumulate along the outer edge and in the center of the storage facility.

The mycotoxin inhibitors to be used should have public research conducted or research publically presented to ensure that the claims presented are valid and unbiased. The companies being evaluated in this experiment are using this and other research findings that they are conducting to produce better products or to know how to best use their product. These companies are already in the development stage of evaluating newer products.

It is possible that the value of mycotoxin inhibitors may vary with different feeding or management conditions. For example we used a dry meal fed diet with weanling pigs. If a swine producer is feeding their feed with water, the enzymes in these or other products might be activated and be more effective than if fed in the dry meal form. The company would be able to address these issues with the swine producer.

With the current 2009 corn crop, the grain should be cleaned and fines removed prior to grinding and mixing into swine diets, as most of the mycotoxin will be located in this portion of the grain.

Wheat and other grains can also be contaminated during the flowering and early milk or “boot” stage. Consequently, the straw from such crops may be contaminated. There is current evidence that at least some of the current 2010 wheat crop may be contaminated with DON.

Stored corn should be dried to a minimum of 14% moisture and aerated frequently so that the mycotoxins will not continue to develop in the bins. When removing grain from the bin, try and remove corn in large batches so as not to isolate “hot spots”.

Weanling pigs and reproducing animals should be fed better corns as they are more sensitive to mycotoxins and these production phases will more readily influence pig profitability. Older pigs, particularly grower finisher pigs appear to be able to tolerate higher levels of DON.

The use of other grains or ingredients free from mycotoxin contamination should be considered in current diet formulas. But they should be screened for mycotoxins.

It is important that when current storage facilities are emptied that they be thoroughly cleaned and a fungicide applied before new corn is added.

Table 1. Project participants and appropriate pig experimental details

Institution	Project leader	Date Started	Weaning age, days	Weaning wt., lb.	Pen spaces ft <sup>2</sup> /pig	No. Pigs	Pigs per pen	Feeder holes per pen
Kansas State University	J. Nelszen	3/11/10	21	14.3	3.8	80	5	3
Michigan State	G. Hill	4/23/10	22	17.8	4.8	80	5	3
OARDC, Western Branch	S. Moeller	2/5/10	25	18.1	3.2	80	5	8
Ohio State University	D. Mahan	2/18/10	17	13.7	4.0	80	5	4
Purdue University	L. Adeola	2/22/10	18-23	13.5	9.6	80	5	1
South Dakota State University	C. Hostetler	2/25/10	21	14.5	7.1	48	3	3
University of Arkansas	C. Maxwell	2/16/10	19	14.6	3.9	80	5	2
University of Kentucky	M. Lindemann	4/29/10	17-21	14.5	4.0	64	4	4
University of Illinois	H. Stein	2/24/10	19	11.9	4.0	64	4	5
University of Minnesota	S. Baidoo	2/16/10	18	13.8	6.6	96	3	3
University of Missouri	M. Carlson	4/3/10	21	14.8	4.0	72	3	4
Virginia Tech	M. Estienne	2/18/10	21	17.5	4.8	80	5	4

Table 2. Composition of basal diet (% , as fed basis)

Ingredient	Days of feeding	
	0 – 10 day <sup>a</sup>	10 – 31 day <sup>b,c</sup>
Corn	41.70	55.85
Soybean meal, 48%	14.25	26.00
Soy Protein Concentrate	3.00	7.00
Dried Whey	15.00	0.00
Plasma Protein	6.00	0.00
Blood meal, pork	0.00	1.00
Fishmeal	6.00	0.00
Lysine	0.20	0.20
DL Methionine	0.20	0.20
Corn starch	0.00	1.00
Lactose	10.00	4.00
Fat, choice white grease	1.00	1.00
Dicalcium Phosphate	0.90	1.40
Limestone	0.55	1.00
Trace mineral premix	0.20	0.20
Salt	0.25	0.40
Zinc oxide, 72% Zn	0.25	0.25
Vitamin premix	0.25	0.25
Mecadox	0.25	0.25
Mycotoxin inhibitor <sup>1</sup>	0.00	±

<sup>1</sup>Mycotoxin inhibitor product added at the expense of corn starch. The products were added only in the treatment test diets fed from 10 to 31 days post weaning.

<sup>a</sup>Corn analyzed 1.9 ppm vomitoxin; < 0.50 ppm T-2 toxin; <0.50 ppm zearalenone (analysis by HPLC).

<sup>b</sup>Corn analyzed 2.0 ppm vomitoxin;< 0.50 ppm T-2 toxin; < 0.50 ppm zearalenon (analysis by HPLC).

<sup>c</sup>Corn analyzed 7.0 ppm vomitoxin; < 0.50 ppm T-2 toxin, < 0.50 ppm zearalenone (analysis by HPLC)

Table 3. Effect of mycotoxin inhibitors added to vomitoxin (DON) contaminated corn and fed to weaning pigs

Item	Corn				Test Corn (2.0 ppm DON)				Test Corn (7.0 DON)				SEM
	Product:	None	Defusion	Integral	Biofix	None	Defusion	Integral	Biofix	None	Defusion	Integral	
	Added/ton; lb.:	0	10	4	8	0	10	6	8				
	Cost/Ton, \$:	0	10.00	11.60	22.32	0	10.00	17.40	22.32				
No. of replicates		27	27	27	27	27	27	27	26				
No. of pigs		113	113	113	113	113	113	113	113				
No. pigs removed (10-31 day)		1	2	0	0	2	0	0	0				
Pig weight, lb.													
Weaning		14.7	14.7	14.8	14.7	14.9	14.8	14.8	15.1				
Start of test, 10 d		18.6	18.7	18.8	18.5	18.6	18.8	18.4	19.4				
Final weight, 31 d		39.8 <sup>a</sup>	41.7 <sup>b</sup>	39.4 <sup>a</sup>	39.7 <sup>a</sup>	34.8 <sup>c</sup>	39.7 <sup>d</sup>	34.1 <sup>c</sup>	33.8 <sup>c</sup>				
Pre test period (0 – 10 d) <sup>1</sup>													
Dietary DON level, ppm		0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80				
ADG, lb.		0.35	0.40	0.39	0.39	0.38	0.39	0.38	0.39				
ADFI, lb.		0.48	0.50	0.49	0.49	0.49	0.51	0.48	0.51				
Test period (10-31 d)													
Dietary DON level, ppm		1.0	1.0	1.0	1.0	3.9	3.9	3.9	3.9				
ADG, lb.													
10 – 17 day		0.71 <sup>a</sup>	0.86 <sup>b</sup>	0.73 <sup>a</sup>	0.74 <sup>a</sup>	0.39 <sup>c</sup>	0.73 <sup>d</sup>	0.42 <sup>c</sup>	0.39 <sup>c</sup>				
17 – 24 day		1.04	1.08	0.97	0.99	0.83 <sup>c</sup>	1.03 <sup>d</sup>	0.77 <sup>c</sup>	0.76 <sup>c</sup>				
24 – 31 day		1.31 <sup>a</sup>	1.42 <sup>b</sup>	1.35 <sup>a</sup>	1.36 <sup>a</sup>	1.11 <sup>c</sup>	1.33 <sup>d</sup>	1.07 <sup>c</sup>	1.06 <sup>c</sup>				
10 – 31 day		1.02	1.09	0.99	1.03	0.75 <sup>c</sup>	1.01 <sup>d</sup>	0.80 <sup>c</sup>	0.74 <sup>c</sup>				
ADFI													
10 – 17 day		0.99 <sup>a</sup>	1.13 <sup>b</sup>	1.01 <sup>a</sup>	1.07 <sup>ab</sup>	0.71 <sup>c</sup>	0.99 <sup>d</sup>	0.69 <sup>c</sup>	0.70 <sup>c</sup>				
17 – 24 day		1.52	1.57	1.41	1.45	1.15 <sup>c</sup>	1.45 <sup>d</sup>	1.08 <sup>c</sup>	1.04 <sup>c</sup>				
24 – 31 day		1.95 <sup>a</sup>	2.13 <sup>b</sup>	1.94 <sup>a</sup>	1.99 <sup>a</sup>	1.64 <sup>c</sup>	1.98 <sup>d</sup>	1.56 <sup>c</sup>	1.61 <sup>c</sup>				
10 – 31 day		1.50 <sup>a</sup>	1.60 <sup>b</sup>	1.46 <sup>a</sup>	1.52 <sup>a</sup>	1.19 <sup>c</sup>	1.49 <sup>d</sup>	1.16 <sup>c</sup>	1.16 <sup>c</sup>				
Feed/Gain 10 – 31 d		1.48	1.46	1.46	1.49	1.60 <sup>c</sup>	1.46 <sup>d</sup>	1.54 <sup>c</sup>	1.64 <sup>c</sup>				

<sup>a, b</sup> Means with different superscripts on the 1.0 ppm diet differed (P < 0.05).

<sup>c, d</sup> Means with different superscripts on the 3.9 ppm diet differed (P < 0.05).

<sup>1</sup> The pretest period involved feeding a common diet without the mycotoxin inhibitor products added. A total of 2 pigs were removed during the pre test period because of unthriftness.

Table 4. Effect of mycotoxin inhibitor products added to vomitoxin (DON) contaminated corn fed to light weight weanling pigs

Item	Corn				Test Corn (2.0 ppm DON)				Test Corn (7.0 ppm DON)				SEM	
	Product:	None	Defusion	Integral	Biofix	None	Defusion	integral	Biofix	None	Defusion	integral		Biofix
No. of replicates	Added/ton, lb.:	0	10	4	8	0	10	6	8	0	10	6	8	-
No. of pigs		29	29	29	29	29	29	29	29	29	29	29	29	-
Weaning weight, lb		12.4	12.5	12.5	12.5	12.5	12.4	12.5	12.4	12.5	12.4	12.5	12.4	-
Test period (10 – 31 d)														
Dietary DON level, ppm		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	3.9	3.9	3.9	3.9	-
Pig weight, 10 d		15.6	15.5	15.8	15.2	15.4	15.5	15.8	15.3	15.4	15.5	15.8	15.3	0.3
Final weight, 31 d		34.3 <sup>a</sup>	37.0 <sup>b</sup>	34.5 <sup>a</sup>	33.6 <sup>a</sup>	28.3 <sup>c</sup>	33.3 <sup>d</sup>	28.2 <sup>c</sup>	27.6 <sup>c</sup>	28.3 <sup>c</sup>	33.3 <sup>d</sup>	28.2 <sup>c</sup>	27.6 <sup>c</sup>	0.9
ADG, lb.														
10 – 17 day		0.60 <sup>a</sup>	0.78 <sup>b</sup>	0.60 <sup>a</sup>	0.66 <sup>a</sup>	0.32 <sup>c</sup>	0.60 <sup>d</sup>	0.31 <sup>c</sup>	0.29 <sup>e</sup>	0.32 <sup>c</sup>	0.60 <sup>d</sup>	0.31 <sup>c</sup>	0.29 <sup>e</sup>	0.03
17 – 24 day		0.87 <sup>a</sup>	1.06 <sup>b</sup>	0.87 <sup>a</sup>	0.89 <sup>a</sup>	0.62 <sup>c</sup>	0.88 <sup>d</sup>	0.65 <sup>c</sup>	0.60 <sup>c</sup>	0.62 <sup>c</sup>	0.88 <sup>d</sup>	0.65 <sup>c</sup>	0.60 <sup>c</sup>	0.05
24 – 31 day		1.23	1.31	1.18	1.13	0.94 <sup>c</sup>	1.12 <sup>d</sup>	0.95 <sup>c</sup>	0.88 <sup>e</sup>	0.94 <sup>c</sup>	1.12 <sup>d</sup>	0.95 <sup>c</sup>	0.88 <sup>e</sup>	0.04
10 – 31 day		0.88	1.00	0.87	0.89	0.60 <sup>c</sup>	0.87 <sup>d</sup>	0.62 <sup>c</sup>	0.60 <sup>c</sup>	0.60 <sup>c</sup>	0.87 <sup>d</sup>	0.62 <sup>c</sup>	0.60 <sup>c</sup>	0.04
ADFI, lb.														
10 – 17 day		0.79 <sup>a</sup>	0.99 <sup>b</sup>	0.82 <sup>a</sup>	0.90 <sup>a</sup>	0.67 <sup>c</sup>	0.80 <sup>d</sup>	0.60 <sup>c</sup>	0.55 <sup>e</sup>	0.67 <sup>c</sup>	0.80 <sup>d</sup>	0.60 <sup>c</sup>	0.55 <sup>e</sup>	0.05
17 – 24 day		1.31	1.40	1.19	1.27	0.86 <sup>c</sup>	1.28 <sup>d</sup>	0.88 <sup>c</sup>	0.93 <sup>e</sup>	0.86 <sup>c</sup>	1.28 <sup>d</sup>	0.88 <sup>c</sup>	0.93 <sup>e</sup>	0.05
24 – 31 day		1.74	1.76	1.73	1.69	1.35 <sup>c</sup>	1.69 <sup>d</sup>	1.41 <sup>c</sup>	1.55 <sup>e</sup>	1.35 <sup>c</sup>	1.69 <sup>d</sup>	1.41 <sup>c</sup>	1.55 <sup>e</sup>	0.05
10 – 31 day		1.39	1.44	1.33	1.41	1.04 <sup>c</sup>	1.39 <sup>d</sup>	1.08 <sup>c</sup>	1.16 <sup>e</sup>	1.04 <sup>c</sup>	1.39 <sup>d</sup>	1.08 <sup>c</sup>	1.16 <sup>e</sup>	0.06
Feed/gain ratio														
10 – 31 day		1.59	1.44	1.56	1.62	1.77 <sup>c</sup>	1.58 <sup>d</sup>	1.81 <sup>c</sup>	2.08 <sup>e</sup>	1.77 <sup>c</sup>	1.58 <sup>d</sup>	1.81 <sup>c</sup>	2.08 <sup>e</sup>	0.08

<sup>a, b</sup> Means within the 4.0 DON corn treatment groups differed (P < 0.05).<sup>c, d, e</sup> Means within the 7.0 DON corn treatment groups differed (P < 0.05).



Table 5. Effect of mycotoxin inhibitors added to vomitoxin (DON) contaminated corn and fed to heavy weight weaning pigs

Item	Corn Test Corn (2.0 ppm DON)				Corn Test Corn (7.0 ppm DON)				SEM
	Product: None	Defusion	Integral	Biofix	Product: None	Defusion	Integral	Biofix	
	Added/ton; lb.: 0	10	4	8	Added/ton; lb.: 0	10	6	8	
No. of replicates	7	7	7	7	7	7	7	7	-
No. of pigs	33	33	33	33	33	33	33	33	-
Weaning weight, lb	17.2	17.2	17.2	17.3	17.2	17.5	17.4	17.1	
Test period (10 – 31 day)									
Dietary DON level, ppm	1.0	1.0	1.0	1.0	3.9	3.9	3.9	3.9	-
Pig weight, lb. 10 d	21.3	21.6	21.6	21.2	21.5	22.0	21.5	21.5	0.5
Final weight, lb. 31 d	45.1	46.2	44.5	44.5	39.5 <sup>c</sup>	44.5 <sup>d</sup>	39.9 <sup>c</sup>	39.3 <sup>c</sup>	1.40
ADG, lb.									
10 – 17 day	0.85	0.91	0.80	0.80	0.44 <sup>c</sup>	0.78 <sup>d</sup>	0.60 <sup>c</sup>	0.44 <sup>c</sup>	0.05
17 – 24 day	1.12	1.05	1.07	1.01	0.90 <sup>c</sup>	1.13 <sup>d</sup>	0.86 <sup>c</sup>	0.93 <sup>c</sup>	0.07
24 – 31 day	1.41	1.53	1.41	1.52	1.23	1.30	1.18	1.18	0.08
10 – 31 day	1.08	1.11	1.04	1.07	0.83 <sup>c</sup>	1.03 <sup>d</sup>	0.86 <sup>c</sup>	0.82 <sup>c</sup>	0.05
ADFI, lb.									
10 – 17 day	1.13	1.18	1.16	1.15	0.75 <sup>c</sup>	1.02 <sup>d</sup>	0.82 <sup>c</sup>	0.80 <sup>c</sup>	0.05
17 – 24 day	1.62	1.67	1.60	1.62	1.17 <sup>e</sup>	1.61 <sup>d</sup>	1.30 <sup>c</sup>	1.26 <sup>c</sup>	0.08
24 – 31 day	2.20	2.30	2.08	2.26	1.94	2.01	1.83	1.74	0.11
10 – 31 day	1.61	1.63	1.55	1.61	1.29 <sup>c</sup>	1.51 <sup>d</sup>	1.34 <sup>c</sup>	1.22 <sup>c</sup>	0.08
Feed/gain ratio									
10 – 31 day	1.46	1.48	1.50	1.49	1.53	1.48	1.47	1.50	0.03

<sup>c, d</sup> Means within the 7.0 DON corn treatment groups differed ( $P < 0.05$ ).

# NIR of feedstuffs and enhancement of NIR prediction of nutrient availability

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## Summary

Cereal grains comprise 60-80 percent of diets fed to high producing livestock, such as swine. Results from large integrated research projects in Australia show that the available energy content (MJ/kg) and available energy intake (MJ/d) of cereal grains incorporated into diets vary widely between and within grain species and animal types. The variation in energy availability between batches of grains can have a large impact on the efficiency of livestock production, time taken for animals to reach market specifications and profitability of livestock enterprises. Similarly, the lysine content and availability of lysine from oilseed meals, such as canola and soybean, can vary widely with batch of seed and oil extraction procedures. Current methods for assessing the energy value of cereal grains or the availability of lysine within oilseed meals are inaccurate or slow. However, near infra-red (NIR) spectra from whole grain and oilseed meal samples are being used to measure rapidly the nutritional quality of these ingredients for livestock. Several of the NIR calibrations developed are being made available to the grains, livestock, feed milling, oilseed crushing and associated industries.

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## Introduction

Cereal grains are fed to animals as a source of energy because of their high starch content and often represent 60-80 percent of the diet for high producing livestock. Similarly, oilseed meals are used frequently in the diets for animals to satisfy their requirements for essential amino acids and total nitrogen. There is large variation across cereal species, cultivars, individual grain samples and animal types in the amount of energy made available to animals from grains following ingestion (Black, 2007). Variation in processing methods and temperature applied to oilseeds during the oil extraction process can markedly affect the availability for animals of essential amino acids, particularly lysine (Newkirk and Classen, 2002). This wide variation in energy and amino acid availability between specific batches of grain and oilseed meals can have marked effects on the efficiency of production, time taken for animals to reach market specifications and profitability of enterprises. Rapid methods for measuring the nutritional quality of grains and oilseed meals are

needed to improve the selection of ingredients and accuracy of diet formulation for the intensive livestock industries.

## Need for new rapid methods for measuring grain and oilseed meal quality

Provided other nutrient requirements are satisfied, the rate of animal production is driven by the intake of available energy (MJ/d), whereas the efficiency of production (kg product/kg feed) is driven primarily by the available energy content of the diet (MJ/kg). Consequently, both measures of energy (MJ/d and MJ/kg) are needed to fully describe the energy value of a grain for animals. Similarly, the total amount of essential amino acids and their availability to animals must be known to fully describe the amino acid value of oilseed meals. Current methods used for assessing the energy value of cereal grains (mean book values, test weight (kg/hl), percent screenings, values calculated from gross composition measurements) do not represent well

either their available energy content (MJ/kg) or available energy intake (MJ/d) when incorporated into diets. Similarly, laboratory methods for assessing the availability of amino acids, particularly lysine, are complex and time consuming, and are rarely used to assess the available amino acid content of individual batches of oilseeds. Consequently, as outlined in this paper, near infra-red (NIR) spectra are being used to develop calibrations for measuring rapidly the energy content, available energy intake index and chemical composition for cereal grains for several livestock types, and for assessing the total and available lysine content of canola and soybean oilseed meals.

## Variation in the energy content of cereal grains

In several large integrated projects within Australia, over 3500 cereal grains (wheat, barley, oats, triticale and sorghum) with a wide range in chemical and physical characteristics have been collected from germplasm archives, plant breeders, farmers and selected because of drought, frost damage or pre-harvest germination (Black, 2008). Over 350 grains selected on variation in NIR scans and *in vitro* fermentation/digestion assays were fed to sheep, cattle, pigs, broilers and layers and 40 grain samples were offered across all animal types. The grains were dry rolled for ruminants and cold pelleted before feeding to pigs and poultry. The energy from grains made available following digestion was measured in all animal types and voluntary intake was measured in cattle, pigs, broilers and layers. A comprehensive chemical and physical analysis was conducted on all grains fed to animals.

The range in available energy content (MJ/kg DM) following digestion of cereal grains fed to different animal types is presented in Table 1. The range tended to be large (3-4 MJ/kg DM) for pigs and poultry offered wheat, barley, triticale and oats, but less (~1 MJ/kg DM) for sorghum. In contrast, the range in available energy content was lower for ruminants than monogastric animals offered wheat, barley and triticale (~1 MJ/kg DM) because of the activity of microbes in the rumen. The variation in available energy content was high for oats fed to ruminants. The range in available energy content for sorghum was closer to monogastric animals for sheep, but not for cattle, where the range was 3 MJ/kg DM, with the high value coming from a waxy

sorghum isolate. Commonly, cattle extract only around 60% of the energy from dry rolled sorghum compared with poultry.

Figure 1 shows the available energy content of grains across animal types when the same grains were fed to cattle, pigs, broilers and layers. Values for pigs tended to be higher than for the other animal types. Values for barley were approximately 1 MJ/kg less than for wheat or triticale and the values for sorghum were higher for pigs and poultry and low for cattle. However, an important observation was that there is little consistent relationship between animal types in the values for individual grains. There were low and negative correlations in available energy content of grains between the animal types (e.g. broilers-pigs, 0.19; broilers-cattle, -0.28; pigs-cattle, 0.21). This observation suggested that individual grains are better digested by one animal type than another.

The experiments also showed that there was no significant correlation between the available energy content of a grain and its intake when incorporated into a diet and fed to pigs, broilers or cattle (Black, 2008). Thus, there were low and negative correlations between available energy content (MJ/kg) and available energy intake (MJ/day) within each animal type (e.g. 0.2 for broilers to -0.1 for pigs) indicating that different characteristics of grains determine digestibility and intake. Similarly, there were low and negative correlations in total available energy intake across animal types (e.g. broilers-pigs, -0.15; broilers-cattle, -0.03; pigs-cattle, 0.12). These low and negative correlations indicate that grains that have high digestibility do not necessarily promote high intakes and that some grains are better than others for providing energy to one animal type and vice versa.

In summary, the results from the experiments offering cereal grains to livestock show:

- The energy value of individual grain samples varies widely between and within grain species and animal types.
- Grains with high digestibility do not necessarily promote high intake and levels of production.
- Individual grain samples are often more suitable for one animal type than another.
- Grains vary widely in their capacity to cause rumen acidosis based largely on the accessibility of rumen microbial enzymes to starch, the rate of starch digestion and the fibre content of the diet.

*Reasons for differences in available energy between grains and animal types.* The energy made available from grain as it passes through the digestive tract depends primarily on the chemical composition of the grain, the physical structure of the endosperm, which influences accessibility of starch to digestive enzymes, and the anatomy of the animal digestive tract (Black 2008). The structure and integrity of the cell walls within the endosperm have a major impact on the available energy content of barley, wheat and triticale samples for different types of animals. These cell walls, composed of a cellulose skeleton impregnated with soluble and insoluble arabinoxylans and  $\beta$ -glucans, must be disrupted by processing, mastication or microbial fermentation to expose starch granules to digestive enzymes from the animal.

Endosperm cell walls have only a minor effect on the overall accessibility of starch from cereal grains for ruminants because they are degraded readily by rumen microorganisms. Thick cell walls take longer to break down than thin walls and slow the rate of starch digestion within the rumen, alter the rate of acid production, may reduce the susceptibility of animals to acidosis and increase starch digestion in the small intestines. Cereal grains with thick cell walls are therefore beneficial to ruminants.

Endosperm cell walls can have a marked effect on the energy value of barley for mono-gastric animals by reducing the contact of amylolytic enzymes with starch granules. These cell walls act either as a physical barrier between the enzymes and starch granules or by increasing the viscosity of the digesta. Endosperm cell walls act more as a physical barrier to the digestion of starch for pigs than for poultry. Grains eaten by birds are subjected to intense grinding in the gizzard and most endosperm cell walls are ruptured. However, pigs appear to rupture few cells during mastication and the availability of energy from cereal grains is increased substantially by fine grinding which exposes the starch to amylolytic enzymes. The increase in digesta viscosity caused by soluble cell wall components in poultry, reduce the diffusion of digestive enzymes through the digesta and reduce the rate of starch digestion. Chain length of soluble non-starch polysaccharide polymers appears to be more important for reducing digestion in poultry than is the total content of soluble non-starch polysaccharides

because of the greater increase in digesta viscosity. Thus, in contrast to ruminants, barley samples with thin and fragile cell walls are most suitable for pigs and poultry.

*Economic importance of differences in energy content of grains.* When the price of wheat is at Australian dollar (AUD) \$250/t, estimates of the money value of a 1 MJ/kg difference in the available energy content of grain range from up to \$18/t for pigs and feedlot cattle, greater than \$30/t for poultry and to around \$15/t for dairy cows fed grain and pasture (Black, 2008). The actual value depends on the base cost of the grain relative to other high and low energy ingredients. Similarly, an increase in apparent metabolizable energy (AME) intake (MJ/d) that stimulates growth rate and results in chickens reaching sale weight one day earlier has been estimated to be worth \$2m/year for a 1 million bird per week broiler operation (Black, 2008). Thus, there is a substantial economic advantage for livestock producers to know the available energy content and the relative available energy intake of individual batches of grain.

*Inadequacy of current methods for measuring grain quality.* The current methods used in Australia to measure grain quality for trading grains for livestock are crude protein content, test weight (kg/hl) and screenings percent being the percentage of grain that is <2.2 mm. The protein content of a grain fed to intensive livestock is relatively unimportant because it is generally cheaper to add protein concentrates to provide the required amino acids than to use protein in grain. Results from experiments show that neither test weight nor screenings percent are good measures of either available energy content or available energy intake of cereal grains (Black, 2008). For example, Figure 2 shows the relationship between test weight and digestible energy content of grains for pigs compared with the Grain Trade Australia (GTA) threshold values for barley (B), triticale (T), wheat (W) or sorghum (S). Apart from a few grains that were heavily frosted and contained little starch, the GTA threshold values are clearly no guide to the energy value of a grain when expressed as available energy content (MJ/kg) or when expressed as available energy intake (MJ/d, Black, 2008). The lack of a close relationship is because the internal morphology of the grain is more important for determining its available energy content for animals than its packing weight in a set volume.

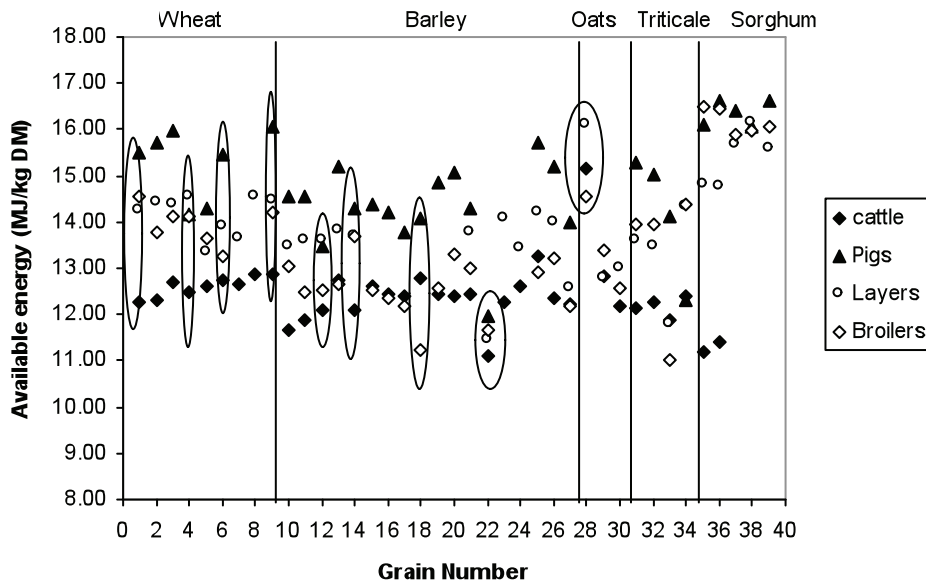


Figure 1. Available energy content (MJ/kg DM) of individual grain samples fed to different animal types. Circled grains illustrate the range in responses across animal types.

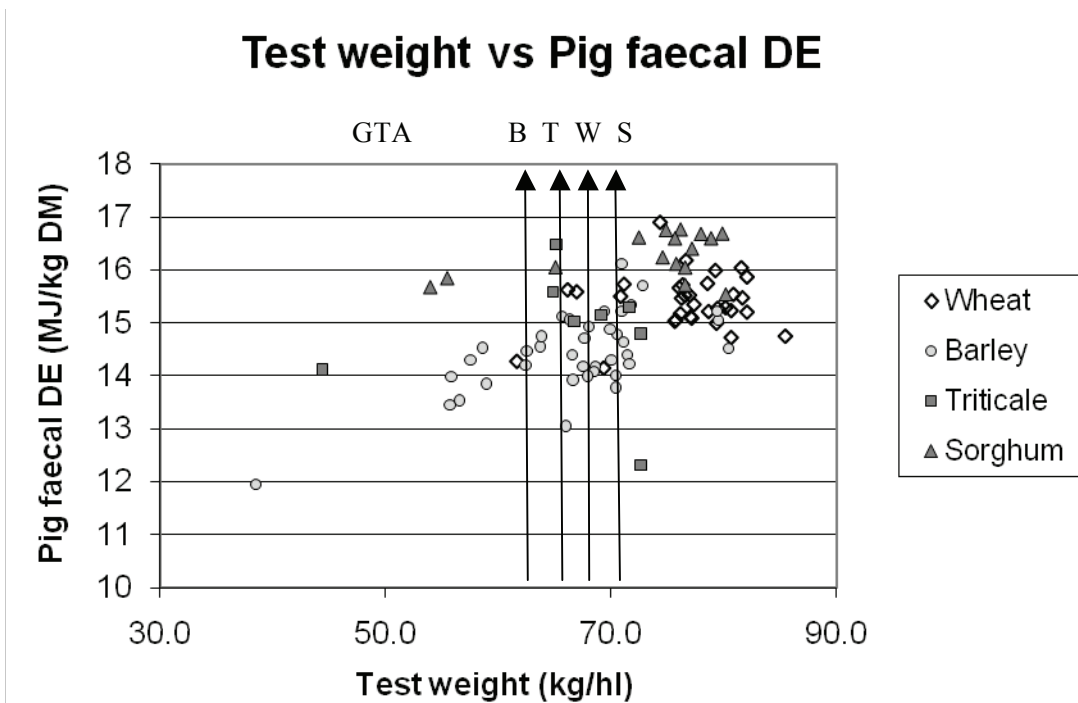


Figure 2. Relationship between test weight and digestible energy (DE) content of the grain for pigs. Grain Trade Australia (GTA) threshold values are shown for barley (B), triticale (T), wheat (W) and sorghum (S).

Table 1. Range obtained in the Premium Grains for Livestock Program for the available energy content of cereal grains following digestion by different animal types.

Animal type	Available energy content (MJ/kg DM) <sup>1</sup>				
	Wheat	Barley <sup>2</sup>	Oats <sup>2</sup>	Triticale	Sorghum
Sheep	12.7-13.7	11.5-13.9	11.2-15.7	12.3-13.4	13.6-14.3
Cattle	12.2-13.1	12.2-13.2	10.8-13.4	12.9-13.2	10.2-13.2
Pigs	12.4-15.0	10.6-14.7	-	12.3-16.5	15.5-16.6
Broilers	12.4-15.6	11.2-13.7	12.6-14.6	11.0-14.6	15.2-16.5
Layers	13.1-17.1	11.0-14.8	12.7-16.4	11.6-14.4	15.5-16.3

<sup>1</sup>ME for ruminants, DE for pigs and AME for poultry.

<sup>2</sup>Naked grain samples included.

### Variation in the available lysine content of canola meal

Approximately 240 samples of canola meal were collected from six commercial oilseed crushing plants in Australia. The samples were produced from both expeller and solvent extraction processes. The cooking temperatures and times for processing were either increased or decreased compared with normal procedures for a small number of samples. The heating of proteins, particularly in the presence of reducing sugars, is known to induce the Maillard reaction, rendering the lysine partially unavailable for metabolism by animals. All samples were scanned through an NIR instrument and 117 samples, which uniformly covered the spectral range, were measured for total and reactive lysine content. Reactive lysine was measured by the method of Moughan and Rutherford (1996) and is considered to represent the lysine available for metabolism by animals.

The total lysine content of the canola meal samples ranged from 16.1 to 23.0 mg/kg as received. Reactive lysine as a proportion of total lysine ranged from 0.66 to near 1.0. These results indicate that there are large differences between individual batches of canola meal in total lysine content and in the availability of lysine. Hence, a rapid method for measuring both total lysine and reactive lysine would lead to more accurate formulation of diets for animals. A similar project determining the variation in the availability of lysine from soybean samples collected from around the world is currently being undertaken within Australia.

### NIR calibrations for measuring grain and oilseed meal quality

The results from the cereal grain projects have been incorporated into NIR calibrations for measuring the energy value of grains for different livestock types and for measuring chemical composition and other grain characteristics. Several of these calibrations have now been made available to the Australian grains and intensive livestock industries through the Pork Cooperative Research Centre, AusScan project. The whole grain NIR calibrations available to commercial enterprises include: cattle metabolisable energy (ME, MJ/kg), sheep ME (MJ/kg), ruminant acidosis index (0-100), broiler AME (MJ/kg), broiler AME intake index (0-100), pig digestible energy (DE, MJ/kg), pig DE intake index (0-100), starch (%), acid detergent fibre (%), neutral detergent fibre (%), total insoluble non-starch polysaccharides (NSP, %), total soluble NSP (%), insoluble arabinoxylans (%),  $\beta$ -glucans (%), hydration capacity (%) and others.

Similarly, NIR calibrations have been developed for measuring the total and reactive lysine contents of canola meals. These calibrations are currently being used by the oilseed processing companies in Australia and, after further validation, are to be made more widely available across the animal and stockfeed manufacturing industries.

The NIR calibration statistics for several of the current NIR calibrations based on whole grain scans are given in Table 2. The table also includes calibration statistics for canola meal samples. The standard error of cross-validation (SECV), which is an indication of the likely precision of prediction, is 0.27 for pig DE. This result means that the calibration can predict with 95% confidence to within

Table 2. NIR calibration statistics for a selection of the calibrations being used by grain, livestock and oilseed crushing industries in Australia.

Calibration	N	Mean	SD	SECV	1-VR	RPD
Cattle, Metabolisable Energy, (MJ/kg DM), <i>ad libitum</i>	96	12.5	0.79	0.34	0.80	2.3
Ruminant, Acidosis Index (0-100)	21	58.3	19.7	10.73	0.70	1.8
Pig, Digestible Energy (MJ/kg as received)	170	13.8	0.71	0.27	0.86	2.6
Pig, Digestible Energy Intake Index (0-100)	60	66.5	15.8	10.85	0.52	1.5
Broiler, Apparent Metabolisable Energy (MJ/kg as received)	180	12.6	1.20	0.45	0.86	2.7
Broiler, Apparent Metabolisable Energy Intake Index (0-100)	184	69.3	10.03	4.22	0.82	2.4
Grain, Acid Detergent Fibre (% DM)	174	6.2	4.0	0.89	0.95	4.5
Grain, Total Starch (% DM)	176	60.9	12.1	3.12	0.93	3.9
Grain, $\beta$ -glucans (% DM)	167	1.8	1.7	0.57	0.89	3.0
Canola meal, Total Lysine (g/kg as received)	113	20.19	1.37	0.65	0.78	2.11
Canola meal, Reactive lysine (g/kg as received)	113	17.96	1.75	0.92	0.73	1.91

$\pm 0.27$  MJ/kg as fed of the measured DE value. The ratio of prediction to deviation (RPD) is an indication of the robustness, or reliability for predicting values for unknown samples, of the calibration. For the pig DE content calibration, PRD was 2.63. NIR experts regard calibrations with RPD values greater than 2.5 as being reliable for predicting values for most unknown samples. The least reliable calibrations from the set commercially available are for ruminant acidosis index and pig DE intake index, whereas the most reliable calibrations are for chemical composition of the grains. NIR calibrations for chemical and physical attributes of grains are generally more robust than those for characteristics measured on animals, such as acidosis index or available energy content, because the standard deviations of the measured values are lower.

The NIR calibrations for pigs, broilers, chemical composition of grains and canola meal are being continually updated and are forecast to become the new method for measuring grain and meal quality and for trading these commodities within Australian. Research is currently underway to include maize in some of the calibrations, so that use of the cereal grain calibrations can be extended to a wider range of countries. The calibrations clearly provide more rapid and accurate estimates of the energy value of individual batches of cereal grains for different livestock types and of the availability of lysine from batches of oilseed meals than the current methods used. The accuracy and robustness of the calibrations is expected to continue to improve through addition of further samples. Nevertheless, those calibrations based on animal studies will not meet Standards Australia's requirements for pricing grains. Under

their regulations, disputes about NIR determined values for a sample must be verifiable using a rapid laboratory analysis. This is not possible when values are determined from complex, long-term experiments using animals. However, the calibrations allow both the grain seller and buyer to know the energy value of a batch of grain and a fair price can be negotiated.

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# Salmonella Transmission, Dissemination, Colonization and Control

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## Summary

Pigs with no previous history of *Salmonella* infection often begin shedding the organism in high concentrations following transportation and lairage. These increases are due to stress and/or rapid infections resulting from contaminated trailers and holding pens. They serve to increase the likelihood of contaminated pork products by amplifying the amount of *Salmonella* brought into the processing facility. One focus of our laboratory is the development of intervention strategies that reduce, or otherwise limit, transportation and lairage associated increases in *Salmonella* shedding by better preparing the animal for the post-farm environment. Phage therapy has proven effective in this regard and we have shown that phage-based treatments can reduce *Salmonella* colonization in pigs under various conditions. Phages have the benefit of being bacteria-specific and non-toxic which could allow administration to animals just prior to processing. In addition, the viruses are easily microencapsulated with minimal effects on viability. This has allowed us to treat multiple animals simultaneously by direct-feeding, which should facilitate the transfer of this technology to the pork industry.

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## Introduction.

There are over 40,000 culture confirmed cases of salmonellosis each year in the United States. While foods of all types have been implicated in outbreaks, *Salmonella* infections often result from the consumption of contaminated meat, poultry or dairy products (CDC 2010). With pork, contamination usually results during processing when the carcass comes in contact with the feces of animal colonized with *Salmonella*. Therefore, reductions in the pathogen loads of animals entering the processing facility can reduce the likelihood of contaminated final products.

Towards this end, many groups have focused on limiting *Salmonella* colonization in the animal while on the farm. Several methods including probiotics, prebiotics, organic acids and other antibacterial compounds have proven effective in at least limiting colonization and shedding. It is quite clear, however, that control of *Salmonella* transmission and contamination of meat products requires a multi-faceted approach with effective intervention

strategies used throughout the production chain.

Transport and lairage are periods in the production chain that are often overlooked in the development of *Salmonella* control strategies. This is problematic as pigs that test negative for *Salmonella* on the farm, often shed the organism in high quantities following transportation and holding. For some time, transport-associated increases in shedding were attributed to stress-induced reactivation of pre-existing infections. In the past decade, several groups have demonstrated that preprocessing increases in *Salmonella* shedding also result, at least in part, from rapid infections from contaminated trailers and/or holding pens (Hurd et al. 2001; Larsen et al., 2004). Regardless of the source of infection, increases in shedding can result in higher concentrations of *Salmonella* entering the processing facility, increasing the risk of end-product contamination.

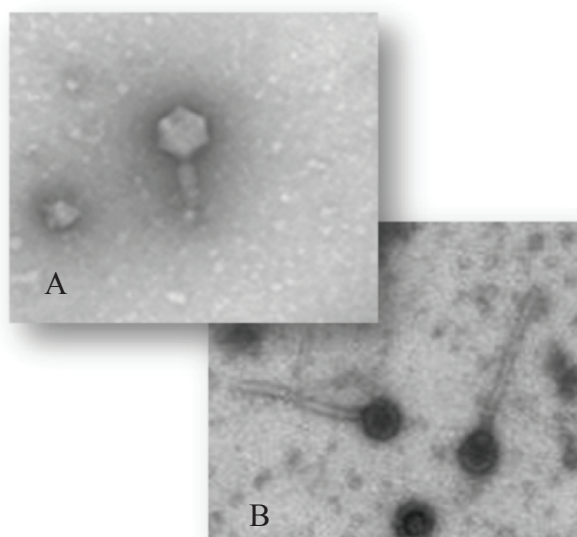
**Phage Therapy.** A focus of our laboratory is

the development of methods that control post-farm transmission of foodborne pathogens by better preparing the animal for the post-farm environment. One technology that has proven effective is phage therapy. Bacteriophages are naturally occurring viruses specific to bacteria. Like all viruses, they are obligate parasites and replicate within the bacterial cell using host machinery and enzymes. In the case of lytic phages, replication is part of a series of events that lead to lysis of the bacterial cell and release of the newly replicated viruses. These viruses are able to then infect neighboring bacterial cells and continue the infection process.

Phage therapy has great potential as a means to control post-farm increases in *Salmonella* shedding. In swine, the time in transport and lairage varies, but averages around 8-10 hours and phage therapy has proven effective at short-term reduction of bacterial colonization. In addition, these viruses are naturally occurring with little to no known toxicity. Therefore, unlike most antimicrobials, phages could be administered to animals just prior to processing (there is currently an FDA-approved phage-based wash for processed meats).

**Phage Isolation and Characterization.** We have a growing library of wild-type, anti-*Salmonella* bacteriophages that were isolated from various sources including poultry litter, swine lagoons and human wastewater treatment facilities. The isolation process involves centrifugation of samples (e.g., influent, raw sludge, transport sludge) to remove heavy sediments and filtration to remove residual bacteria. The filtrate containing virus particles is then enriched by co-culturing with *Salmonella*. The viruses are ultimately isolated and purified by standard plaque assays. All the viruses in our library are tailed phages with icosahedral heads typical of enteric phages. Each virus belongs to one of two families. Those with long, non-contractile tails are Siphoviridae, while those with short, contractile tails are Myoviridae (Figure 1).

**Microencapsulation.** It is often necessary to protect biological therapies prior to delivery to ensure that the live organisms are able to reach the actual sites of infection. This is especially true with treatments given orally if they are required to bypass and survive the harsh environment of the stomach. We have been successful in protecting our phage



**Figure 1. Transmission electron micrographs of wild-type anti-*Salmonella* phages.** A) tailed phage with short, contractile tail representative of Myoviridae; B) tailed phages with long, non-contractile tail representative of Siphoviridae.

cocktails with microencapsulation. In basic terms, microencapsulation involves surrounding the live organism in lipid microspheres. Microencapsulated phages are able to withstand the low pH of the stomach in greater concentrations than naked phages. Upon entering the small intestine, the microspheres are broken down releasing the phages into a more hospitable environment.

The microencapsulation method that we use is a sodium-alginate based method. The process itself does not appreciably affect phage viability. Microencapsulation typically reduces phage titer by one log (e.g., pre-microencapsulation concentration:  $10 \log_{10}$  PFU/mL; post-microencapsulation concentration:  $9 \log_{10}$  PFU/mL), but these decreases are usually the result of dilution during the microencapsulation process. Once microencapsulated, phages remain viable with no drop in titer for up to 14 days at both 4°C and 22°C.

**Preliminary Live Animal Trials.** We have tested the ability of phage therapy to reduce *Salmonella* colonization in pigs under various conditions. Our first animal experiments involved small pigs (~30-40lbs) that were co-inoculated with a microencapsulated phage cocktail consisting of 15 anti-*Salmonella* phages and a non-lethal dose

( $10^7$  CFU) of *Salmonella enterica* Typhimurium. We collected fecal samples periodically from both the pigs and the environment. At six hours, the pigs were euthanized and we collected tonsil, ileal, cecal, mesenteric lymph node and fecal samples. All samples were screened for the presence of the challenge *Salmonella* as well as anti-*Salmonella* phages. In these preliminary experiments, pre-treatment of pigs with the phage cocktail in several cases resulted in a 99.9% reduction in the concentration of *Salmonella* compared to pigs receiving a mock-treatment (Table 1; Wall et al., 2010).

**Simulated Production Setting.** As our ultimate goal is to use phage therapy on market weight pigs just prior to processing, we have also tested our phage treatment on pigs under more production-like settings. For these experiments we used a model intended to mimic a processing facility holding pen. We challenged market weight pigs with *Salmonella*, housed them together in a single pen and allowed them to contaminate the pen via shedding. At three days post-challenge, we administered the phage cocktail orally to a group of *Salmonella*-naïve pigs. Phage treated pigs were then co-mingled with the seeder pigs in the contaminated environment for six to eight hours, a typical time a pig might be in transport and lairage in the United States.

As in previous experiments, we collected fecal samples periodically from both the pigs and the environment. At eight hours, all pigs were euthanized and ileal, cecal, mesenteric lymph node and fecal samples were collected and screened for the challenge organism and anti-*Salmonella* phages. Pre-treating pigs with the phage cocktail prior to their entry into a *Salmonella* contaminated environment significantly (up to 95%;  $P < 0.05$ ) reduced *Salmonella* colonization in various tissues compared to mock-treated pigs, indicating that phage therapy may prove effective at limiting preprocessing increases in *Salmonella* shedding (Table 1; Wall et al., 2010).

**Feed Based Delivery.** In all of our previous experiments, phages were delivered to individual pigs by gavage. This was to ensure that all pigs received a uniform dose of viruses. Individual treatment of pigs, however, is not practical when numerous animals are marketed simultaneously. Our earlier

**Table 1. Percent reduction in *Salmonella* colonization in phage treated pigs under various conditions**

	Ileum	Cecum
<b>Pigs co-administered phage cocktail and <i>Salmonella</i></b>	99.9	99.5
<b>Pigs exposed to <i>Salmonella</i> contaminated environment (simulated holding pen)</b>	90.0	95.0
<b>Pigs administered phage cocktail in the feed</b>	90.0	90.0

experiments demonstrated that microencapsulated phages remained viable at room temperature for up to 14 days, indicating that the phages could be delivered via feed. To test this hypothesis, we included phages in the feed of pigs for five days prior to challenging them with *Salmonella enterica* Typhimurium. Feed-based delivery was equally effective as gavage delivery and reduced *Salmonella* colonization more than 90% in some tissues.

**Expanding the Host Range of Phage-based Treatments.** One limit to phage therapy is specificity. Some phages will only recognize and infect certain species of bacteria, and in some cases, certain serovars within a species. For such narrow spectrum treatments to be effective, a large amount of diagnostic information is usually required. Recently we have focused on creating phage cocktails with increased host ranges. Using USDA Food Safety Inspection Service annual data, we have identified *Salmonella* serovars that are regularly among the most frequently isolated from livestock, humans or both and isolated individual viruses that are can effectively lyse multiple serovars. One such cocktail contains ten phages that together produce titers of over  $10.5 \log_{10}$  PFU/mL on co-cultures of *Salmonella enterica* Typhimurium, Enteritidis and Kentucky (Zhang et al., 2010).

**Future Directions.** There are still challenges to adopting phage therapy as a pre-processing

food safety intervention strategy. Foremost is inconsistency in efficacy. One treatment may produce different results under slightly different conditions. In general, there is very little basic information regarding the viruses that have been incorporated into successful phage cocktails. The phages in our library have only been minimally characterized. We are currently focused on gaining a better understanding on the basic properties of these viruses. These experiments include characterizing growth kinetics (latency periods, burst sizes, etc.), genetics, survivability under various conditions, infectivity, attachment and other virus-host relationships. We are confident that more comprehensive characterization of these viruses will allow us to determine which phages are most effective under different conditions and, in turn, produce more uniform effects. At the same time, a better understanding of the basic properties of the phages will allow us to predict where else phage therapy might be effective and appropriate.

**Acknowledgments.** This research was supported in part by the National Pork Board (#06-167 and #09-191).

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# Overview of Environmental Monitoring Research at Purdue University

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## Summary

As swine facilities grow in size, their potential for creating objectionable odors and increased air emission plumes becomes a greater concern for neighbors, the general public, and regulators. Much of the emitted gases come from the anaerobic decomposition of manure during storage, the release of volatile organic compounds and ammonia immediately after excretion from the animal, land application of manure, and dust generated in the building facilities carrying odors and gasses. Diet manipulation can have a great impact on nutrients excreted as well as odors and gasses emitted from the facility. This report highlights some recent research conducted at the Purdue University 12-room swine environmental building designed to research and address these environmental issues related to diet manipulation and manure management.

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## Introduction

In the past two decades, the pork industry has undergone rapid technological and structural change. The most significant changes have been a decrease in farm numbers, an increase in production facility size, and the movement of large production operations to more rural areas of the country. The number of farms raising hogs declined by 83% from 1965 to 1995 (USDA Report, 1996). Additionally, from 1997 to 2002, the number of farms with swine decreased by 45.7% or an average of over 9% per year during this 5 year time span and has remained fairly stable since (USDA report, 2010). Even though the swine industry has seen record losses of farms, relatively little change in the annual number of pigs raised in the US has occurred between 1965 and 2006, but has experienced a 10-15% increase recently in 2007 that has been maintained through 2009. Unfortunately, animal feeding operations can affect air quality through emissions of odor, odorous gases (odorants), particulates (including biologic particulate matter), volatile organic compounds, and some greenhouse gases (Arogo et al., 2001; Bicudo, et al., 2001; Sweeten, et al., 2001; USDA AAQTF, 2001; and NAS, 2003). Much of the emitted gases come from the anaerobic decomposition of manure during storage, the release of volatile organic compounds and ammonia immediately after excretion from the animal and dust generated in the building facilities from feed delivery systems, animal

movement, and hair and sloughed skin from the animal. New regulatory pressures to meet water and air quality standards for CAFO's (EPA, 2003) and NPDES permit regulations, including the possibility of meeting total maximum daily load (TMDL) of contaminants in the water supply and stricter air quality regulations are placing additional economic and management burdens on pork producers which may lead to further consolidation of the industry.

Much of the public awareness of the potential threat of swine manure to water pollution has been due to a few large operation's having spills. Media attention and activity groups have applied pressure on producers, legislators and regulators for management changes in livestock operations. In many cases, odors, dust and gas emissions from swine units have resulted in nuisance lawsuits and unrealistic regulations not necessarily based on scientific evidence. Residents near operations are concerned about the potential devaluation of their property and the impact of manure and odors on their health and lifestyle. State and local governments are struggling to develop long term land use plans to maintain sufficient land areas for both pork operations with land application of manure and the influx of urban residents into rural areas. Therefore the objectives of this research are to determine the amount of gases, odors and dust emitted from buildings when swine are fed different diets and two manure storage strategies are utilized.

## Methods

To accurately determine nutrient excretion and emissions from a commercial-like facility, the Swine Environmental Research Building (SERB) at Purdue University was constructed. This facility houses 720 pigs in 12 rooms with 6 pens per room and 10 pigs per pen (Figure 1). Each room is divided by a center isle way. Manure is quantitatively collected and stored in a deep pit under each side of the room (3 pens of 10 pigs each). The two manure pits per room are divided by a wall under the central isle way. Pits have enough capacity to store an entire wean-finish period worth of manure. Manure can be sub-sampled using a vacuum driven core sampler, or emptied individually from each pit, mixed and sub-sampled. The building is equipped with a centralized laboratory capable of monitoring gas emissions from each independently ventilated room. The facility allows for a complete nutrient mass balance by room (feed, pigs, manure, and air).

## Results

Research conducted in SERB has compared traditional diets with Low Nutrient Excretion (LNE) diets (Table 1), which have reduced CP, P and Ca contents coupled with supplemental amino acids and phytase. Pigs fed these diets, grew faster on less feed, resulting in better feed efficiencies (Table 2).

Similar to live weight, carcass weights (Table 3) were 3.8 kg heavier ( $P < 0.001$ ) for LNE fed pigs (96.6 kg) compared to CTL fed pigs (92.8 kg). Along with higher carcass weights, LNE fed pigs had an extra 2.2 mm of backfat depth ( $P < 0.001$ ), with no differences ( $P > 0.10$ ) in carcass loin depth, resulting a slight reduction in percent lean (53.97% vs. 53.37%;  $P < 0.001$ ) for LNE fed pigs compared to CTL fed pigs.

Dry matter, N, and P excretion were higher throughout the study for control-fed pigs compared to LNE fed pigs (Figure 2). Pigs fed LNE diets averaged 22.7% less nitrogen and 36.3% less phosphorus in the manure over the wean-to-finish study. Similarly, ammonia and hydrogen sulfide emissions were higher for control-fed pigs, with the difference in air emissions between control and LNE fed pigs increasing as the pigs got older and heavier (Figure 3).

## Implications

The most significant change seen in the swine industry has occurred over the last twenty years. We have seen a shift from many farms producing a limited number of pigs to a small number of large confinement production facilities. New regulatory pressures to meet water and air quality standards for CAFO's and NPDES permit regulations are placing additional economic and management burdens on pork producers, which may lead to further consolidation of the swine industry. Preliminary data presented in this proceedings paper illustrates that feeding low nutrient excretion diets does not have to result in poor animal performance or carcass characteristics to yield significant reductions in gaseous compounds. Pigs fed the low nutrient excretion diets had improvements in average daily gain, feed efficiency, and were approximately 5.0 kg heavier at market than pigs fed control diets. Although backfat thickness was greater for low nutrient excretion fed pigs, there was an increase in carcass yield and total carcass value for the LNE fed pigs. More data needs to be analyzed to determine accurate air emission data, however by reducing emissions from swine facilities; there can be less neighborly concern and more acceptance of the swine industry. Moreover, this data will serve as a modeling tool for producers, extension educators, regulators, consultants, and legislators to plan environmentally sound pork production systems throughout the United States.

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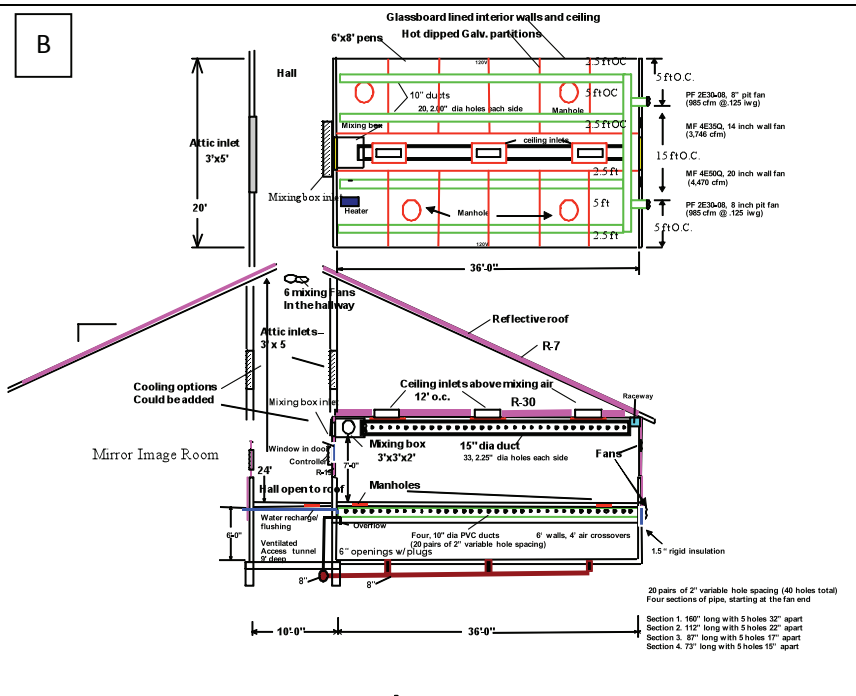
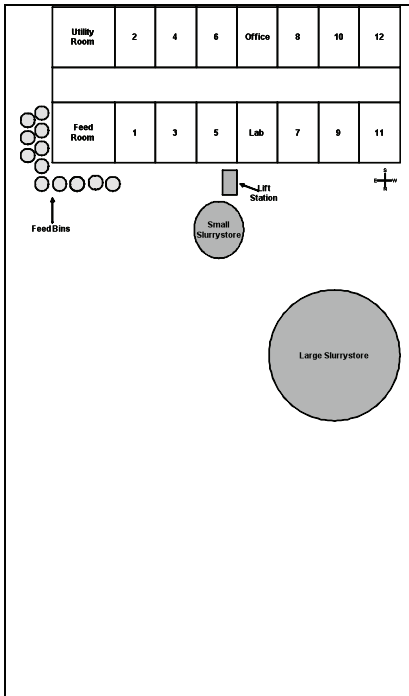
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**Figure 1.** A) Diagram of the overall layout of Purdue’s Swine Environmental Research Building (SERB), including the 12 experimental rooms, feed storage and manure storage. B) Detailed cross-sectional and overhead schematic of SERB.

Table 1. Grower 1 and Finisher 2 diet composition.

Ingredient, %	Grower 1				Finisher 2			
	Gilts		Barrows		Gilts		Barrows	
	Control	LNE <sup>a</sup>	Control	LNE	Control	LNE	Control	LNE
Corn	68.04	67.26	69.77	69.148	84.910	85.980	86.720	87.990
SBM	29.27	25.72	27.53	23.82	13.370	7.930	11.540	5.910
Choice White Grease	0.00	4.00	0.00	4.00	0.000	4.000	0.000	4.000
Calcium Carbonate	0.70	0.94	0.71	0.95	0.660	0.910	0.670	0.920
Dical	1.11	0.74	1.12	0.75	0.510	0.150	0.520	0.170
Vitamin <sup>b</sup>	0.15	0.15	0.15	0.15	0.100	0.100	0.100	0.100
TM <sup>b</sup>	0.09	0.00	0.09	0.00	0.050	0.000	0.050	0.000
Non-Sulfur TM <sup>b</sup>	0.00	0.09	0.00	0.09	0.000	0.050	0.000	0.050
Phytase <sup>c</sup>	0.00	0.083	0.00	0.083	0.000	0.083	0.000	0.083
Salt	0.35	0.35	0.35	0.35	0.250	0.250	0.250	0.250
Lysine-HCL	0.10	0.29	0.10	0.30	0.100	0.330	0.100	0.330
DL-Methionine	0.02	0.10	0.01	0.09	0.000	0.030	0.000	0.020
L-Threonine	0.02	0.12	0.02	0.11	0.000	0.110	0.000	0.100
L-Tryptophan	0.00	0.007	0.00	0.009	0.000	0.030	0.000	0.030
Antibiotic	0.10	0.10	0.10	0.10	0.025	0.025	0.025	0.025
Se 600	0.05	0.05	0.05	0.05	0.025	0.025	0.025	0.025
Total	100	100	100	100	100.00	100.00	100.00	100.00

<sup>a</sup> LNE = Low nutrient excretion diet.

<sup>b</sup> Vitamin premix supplied per kg of diet: Vitamin A, 2425.1 IU; Vitamin D, 242.5 IU; Vitamin E, 17.6 IU; Menadione, 0.80 mg; Vitamin B<sub>12</sub>, 14 µg; Riboflavin, 2.82 mg; d-Pantothenic Acid, 8.81 mg; Niacin, 13.2 mg. TM premix supplied per kg of diet: Iron, 48.5 mg; Zinc, 48.5 mg; Manganese, 6.0 mg; Copper, 4.5 mg; Iodine, 0.18 mg; Selenium, 0.30 mg. A non-sulfur TM premix was used for the LNE diets.

<sup>c</sup> Phytase, Natuphos, 600 PU/kg, BASF, Mt. Olive, New Jersey, USA.



Table 2. Grow-Finish growth performance.

Diet Sex	Control		LNE		Significance, P <		
	B	G	B	G	MSE	Diet	Sex
<i>Overall grow-finish</i>							
ADG, kg/d	0.99	0.95	1.02	0.97	0.003	0.002	0.001
ADFI, kg/d	2.86	2.76	2.60	2.45	0.032	0.001	0.002
G:F	0.35	0.35	0.39	0.40	0.001	0.001	0.81
Final BW, kg	128.74	125.12	133.41	129.06	39.10	0.001	0.002

Table 3. Carcass composition.

Diet Sex	Control		LNE		Significance, P <		
	B	G	B	G	MSE	Diet	Sex
Lean, %	53.46	54.47	52.65	54.09	0.446	0.001	0.001
Yield, %	74.41	74.28	75.90	74.71	3.804	0.02	0.56
Loin depth, cm	6.58	6.61	6.58	6.58	0.050	0.58	0.06
Fat depth, mm	23.23	19.43	26.47	20.73	5.434	0.001	0.001
Hot carcass wt, kg	95.35	92.15	98.91	94.37	27.396	0.001	0.001
Grade premium, \$/kg	0.11	0.13	0.08	0.13	0.001	0.001	0.001
Live value, \$/kg	0.99	1.02	0.97	1.01	0.007	0.23	0.01
Total value, \$/pig	92.77	93.07	94.59	95.34	2.280	0.19	0.74

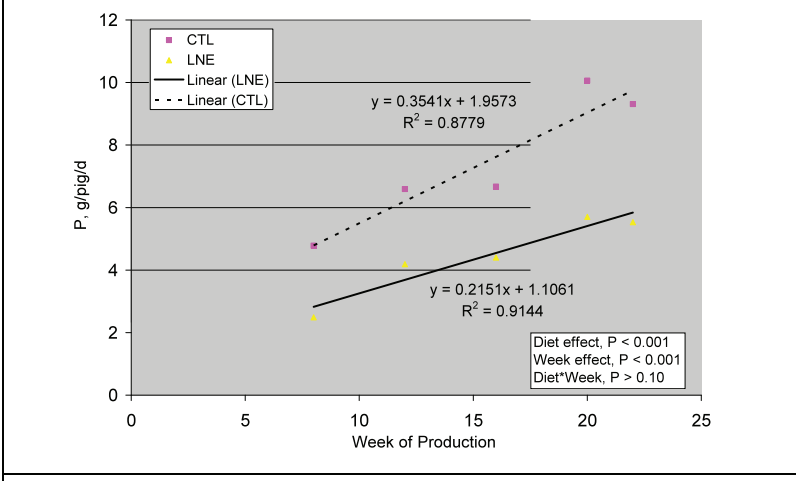
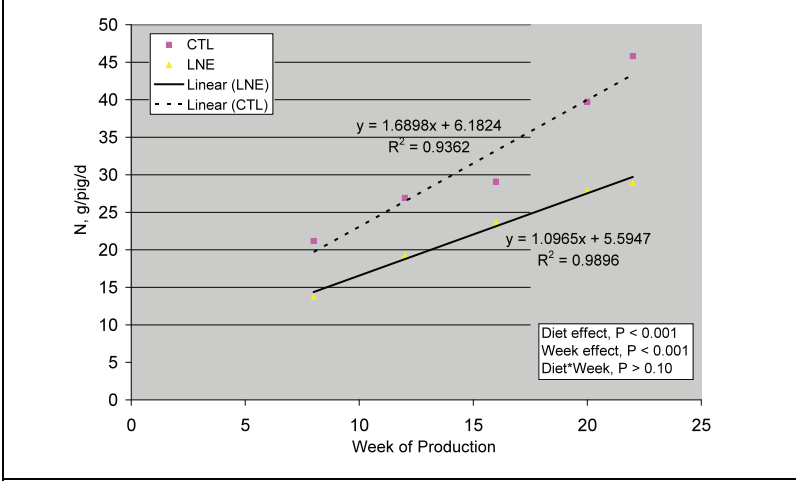
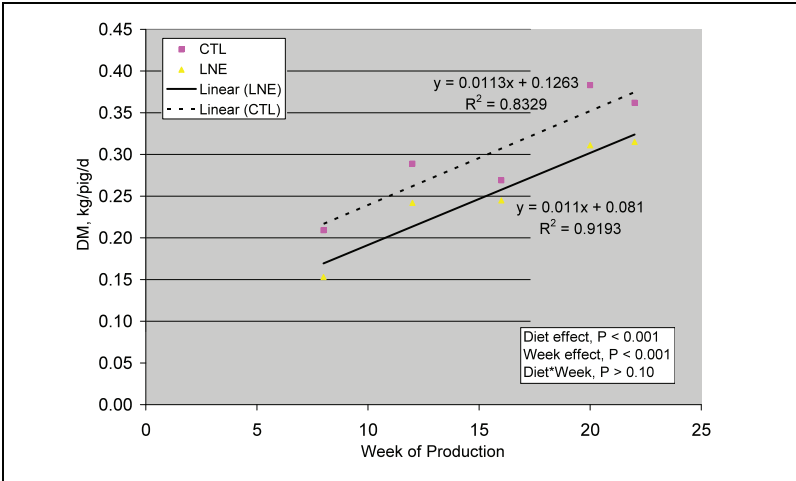


Figure 2. The effects of feeding a LNE diet compared to a control diet on DM, N and P excretion.

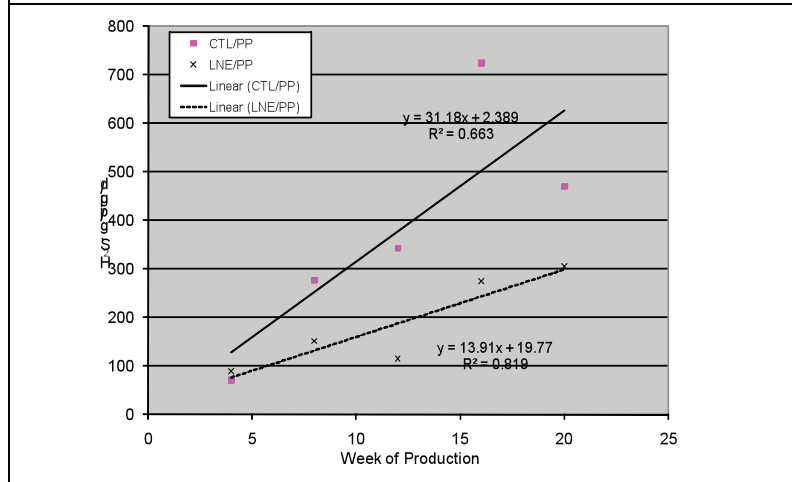
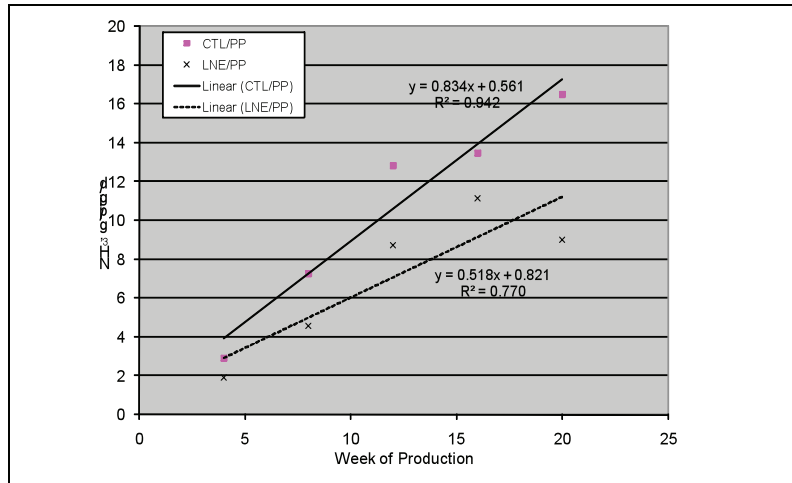


Figure 3. The effects of feeding a LNE diet compared to a control diet on daily ammonia and hydrogen sulfide emissions for pigs reared over a monthly pull plug/recharge manure system.

# Net Energy – Current Status

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## Summary

Energy is the most expensive dietary essential in pig diets, but it receives much less attention in North America than is deserved by its importance. The digestible energy (DE) and metabolizable energy (ME) systems widely used in North America share important shortcomings. More sophisticated systems have been adopted in Europe but North American nutritionists have largely not developed confidence in them. We conducted a multi-year and multi-institutional study to evaluate the European systems under North American conditions, as a first step in moving our industry to more accurate diet formulations and more profitable feeding programs. Our approach was to measure the net energy (NE) value of several ingredients and compare those values to the ones predicted by the European systems. The results suggest caution in use of the current European systems because those systems do not accurately predict our measured values, although in important comparisons they appear to be superior to ME. Our data show that animal factors should be considered in an energy system. We recommend that our values be tested in practical feeding trials, especially our relatively low values for fats and fibrous ingredients, before proceeding to development of a complete new system.

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## Introduction

Energy is the most expensive dietary essential in pig diets, but it receives much less attention in North America than is deserved by its importance. In North America it is usually expressed as either digestible energy (DE) or metabolizable energy (ME). An energy system includes energy requirements and energy contributions of feedstuffs and diets, all expressed in the same units. For example, DE and ME are energy systems.

The DE and ME systems share important shortcomings: stoichiometry suggests that they systematically overvalue fibrous or high-protein feedstuffs and they systematically undervalue fats. These shortcomings seriously limit the precision of formulations the industry needs to ensure high production while limiting costs and environmental

impact. It is increasingly apparent to many practicing nutritionists that these deficiencies in measurement of dietary energy are important to the economics of pig production.

To improve upon DE and ME, it is logical to move to a net energy (NE) system. Three NE or related systems are now available, all developed in Europe. However, none of these systems has gained the confidence of North American swine nutritionists for use in the diets and production systems common in North America. That leaves a damaging void, when considered along with the serious systematic biases of the DE and ME systems.

As we refine energy systems to the NE level, it becomes apparent that expression of an energy value for feeds is theoretically inadequate, because animal factors influence the efficiency of energy use. For example, use of energy substrates to build body fat is more efficient than use of those same substrates to drive protein accretion, and the magnitude of the difference varies among substrates. We have argued

<sup>a</sup>G.L. Allee, J.F. Patience, H.H. Stein, D.Y. Kil, R.B. Hnson, A.D. Bioulieu, F. Ji, and L.L. Stewart led various aspects of the major project described in this paper. Many others contributed to the work.

that the NE value of starch, and therefore corn, is theoretically higher when it is converted to body fat than when it is used to support body protein accretion.

To address this void for North American producers, we have undertaken a multi-year multi-institutional research program designed to develop a North American Swine Energy System, under the leadership of the National Pork Board and the United Soybean Board. This paper summarizes the results of the first 2-year phase of that projected long-term program.

The following characteristics of this project are especially important:

- The pigs were housed, fed and managed as nearly like pigs in commercial North American pig production as is feasible, including free access to feed. This characteristic lends confidence in application of the results in practice. It differs substantially from the respiration calorimetry used in Europe, which houses pigs individually and limits their feed intake.
- Measurements were made over periods of 4 or 5 weeks, in contrast to respiration calorimetry measurements which may cover only a day.
- The experiments were designed to test specific questions important under North American conditions. The broad-based approach used in development of the European systems is powerful when applied to broad comparisons among feed ingredients, but may not precisely reflect the relative value of specific ingredients.

## Objectives

To determine whether any of three European energy systems is adequate for use in North American conditions, and whether a sound energy system must consider animal factors (e.g. protein vs. fat deposition).

## Overview of Experimental Methods

We conducted a series of 16 experiments strategically designed to address issues of concern; 6 measured the operational maintenance requirements, 8 directly measured NE of ingredients, and 2 addressed the use of low-protein diets with aggressive use of crystalline amino acids. All projects used a comparative slaughter method to

determine NE by measuring the amount of energy retained in the pig's body during the test period. The 703 pigs used in these experiments were killed and ground for measurement of body energy content. All measurements were made at each of two stages of growth, beginning at about 50 lb. and 200 lb. body weight, to assess differences in energy values due to age and/or composition of gain. The NE content of the diet is the energy retained in the body plus the NE requirement for the work of the body other than growth, a value we call operational maintenance. The critical step in this research is comparison of our measured NE values to the values predicted by the European systems.

Initial energy content of the body was determined by harvesting and analyzing 16 representative pigs at the beginning of each experiment. Then experimental diets were fed to 8 individually-penned pigs per treatment for a period of 4 weeks for growing pigs or 5 weeks for finishing pigs, and those pigs were harvested for analysis. The amount of energy retained in the body during the experimental feeding period was calculated by difference.

The NE values of target ingredients were determined by the substitution method. The ingredients measured were:

- Corn (in low-fat and high-fat diets)
- Soybean meal
- Low-oligosaccharide soybean meal
- Soy oil (5 or 10% of the diet)
- Choice white grease (10% of the diet)
- Soy hulls
- Wheat midds

All test ingredients were analyzed for proper prediction of NE content by the European systems. Nutrient digestibility was measured weekly from grab samples of feces.

## Comparison of Results to European Systems

The European energy systems considered are the NE systems from France (designated INRA; Noblet et al., 1994) and the Netherlands (designated CVB; CVB, 1994) and the Potential Physiological Energy system from Denmark (designated PPE; Boisen, 2007). Our approach was to compare the energy values predicted by these systems and by the ME system to the values we measured directly. We

are acutely aware that our values are not measured without error, and we take that fact into account in drawing conclusions.

All of the European systems calculate the energy value using prediction equations. The NE systems base predictions on digestible nutrient levels; the INRA system offers several related equations that use digestible nutrient data in different forms. These equations allow consideration of variation of ingredients. INRA also offers tabulated NE values of ingredients (Sauvant et al., 2004), and we have considered these also in some cases to avoid reliance on our measures of digestibility. The PPE system bases predictions on specialized laboratory measurements.

Energy values predicted by the European systems are considerably higher than our measured NE values for both diets (Tables 1 & 2) and ingredients (Tables 3 & 4). Part of the difference is use of a higher estimate of the operational maintenance requirement in the European systems than in ours; that value is important because it is added to the measured energy retention to determine the NE of a diet. It may be appropriate to apply different values for the maintenance requirement than the one we estimated, but that would not change the overall conclusions of our work. Besides differences in maintenance requirement estimates, there are also methodological differences that may be important.

The energy consumed by the pig and not eliminated in feces or urine (ME) is either retained in the body, primarily in the form of protein and fat, or it is released as heat. Our comparative slaughter method measured the amount of energy retained by pigs over a period of several weeks. Conversely, the respiration calorimetry upon which the European systems are based first estimates the amount of heat released, calculated from oxygen consumption and carbon dioxide production, and subtracts that from ME to determine retained energy. Measurements are made on individual pigs housed in the calorimeter chambers for usually 1 day.

Others (Baldwin and Bywater, 1984; McCracken and McAllister, 1984) have reported that direct estimates of energy retention by comparative slaughter are lower than the indirect estimates by respiration calorimetry, consistent with our results. We need to identify and quantitate the specific reasons for the difference. Factors likely involved include differences in thermal stresses, social/

psychological stresses, subclinical disease challenge, energy use for physical activity, feeding level and feed wastage among others.

Although the systematic overestimation of energy values by the European systems is of concern, it would not interfere substantially with use of those systems for evaluation of the energy contribution of feedstuffs, as long as the target dietary energy levels are consistent with the assumed levels in feedstuffs. The relative values of feedstuffs are more important than the absolute levels. Therefore, our focus on evaluation of the European systems is on how well they predict the quantitative relationships among diets and ingredients that we measured.

We have compared our measured NE values to the ones predicted by the European systems in several ways, but the most instructive addresses the ratios of energy values among ingredients (Tables 5 and 6). For this purpose we used corn as the standard to which other ingredients were related. Our value for corn was the mean of the values measured in low-fat and high-fat diets. Salient observations are:

- **Soybean meal:** The ME system overestimates the value of soybean meal relative to corn, as expected. The empirically-derived French and Dutch systems make an important correction in the appropriate direction. The prediction equations from those systems, using our measures of digestibility, make a correction much smaller than our data suggest appropriate, but the INRA tabular values show a correction substantially larger than shown by our data. The Danish system agrees well with our measurements in this comparison.
- **Fats:** The ME system is perceived to underestimate the energy value of fats relative to corn, and the three European systems make corresponding adjustments to varying degrees. Our data show best agreement with the ME system, when evaluated by the ratio of energy value of fats to that of corn. The European systems do not distinguish among types of fat, but our data suggest differences between relatively saturated and relatively unsaturated fats.
- **Fiber:** As expected, ME overvalues fiber compared to our measurements. The European systems are closer, but overestimate our ratios in most cases.

Since the completion of the major project, we have taken initial steps to test in separate experiments the low NE values for fat and fibrous materials that we measured and the difference between fat sources that we found. First, we measured the NE value of DDGS, which contains a high level of both fat and fiber (Gutierrez et al., 2009). Our NE values for DDGS were substantially lower than predicted by the INRA system, consistent with the results of our major project. Second, we compared the impacts of several fat sources on the growth performance and backfat thickness of finishing pigs (Liu et al., 2010a,b). The results provide modest support for the difference in NE values between choice white grease and soy oil that we found in the major project.

#### *Consideration of Animal Factors*

Certain characteristics of the animal, such as its maturity or the composition of its gain, theoretically affect the efficiency of energy use and therefore the NE values. Our strongest evidence concerning the importance of animal factors in an energy system is in the comparison of our values for growing versus finishing pigs. Our measured values for finishing pigs are generally greater than those for growing pigs, presumably because of greater digestive capacity and the deposition of a greater proportion of fat (more efficient) to protein (less efficient). However, as described above with regard to the European systems, that difference does not negate usefulness in an energy system. The key question is whether the quantitative relationships among ingredients are consistent across stages of growth. The results (Table 7) suggest they are not. Specifically, the fats fed at 10% of the diet stand out, in contrast to the other ingredients evaluated, as not showing higher NE value for finishing pigs than for growers. This phenomenon can also be seen in corresponding values in Tables 5 & 6 and, to a lesser degree, in the earlier tables. This inconsistency of relationship among ingredients between growing and finishing pigs indicates a need for consideration of animal factors in an energy system.

## **Conclusions and Recommendations**

- We recommend caution in use of the current European systems because those systems do not

accurately predict our measured values, although in important comparisons they appear to be superior to ME.

- Our data show that animal factors should be considered in an energy system.
- We recommend further exploration of the utility of the Danish Potential Physiological Energy system for use in North America, perhaps with modifications.
- We recommend that our values be tested in practical feeding trials, especially our relatively low values for fats and fibrous ingredients, before proceeding to development of a complete new system.

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**Table 1. Net energy values (Mcal/kg dry matter) of diets measured in the present project and predicted values of those diets from other energy systems: Growing pigs<sup>a</sup>**

Experiment	Diet	Present	INRA- calc	CVB	PPE	ME (NRC)
Corn	Low-fat basal	2.02	2.54	2.59	2.21	3.58
	Low-fat corn	2.04	2.56	2.73	2.31	3.64
	High-fat basal	2.19	2.91	2.94	2.41	3.94
	High-fat corn	2.13	2.84	2.88	2.48	3.92
Soybean meal	Basal	2.22	2.64	2.72	2.46	3.70
	Regular	2.06	2.57	2.65	2.32	3.72
	Low-oligosaccharide	2.15	2.55	2.68	2.28	3.72
Fat	Basal	2.13	2.56	2.55	2.24	3.69
	Soy oil, 5%	2.25	2.88	3.07	2.48	3.92
	Soy oil, 10%	2.42	3.12	3.15	2.72	4.16
	Choice white grease	2.53	3.13	3.14	2.76	4.11
Fiber	Basal	2.26	2.34	2.32	1.52	3.77
	Soy Hulls	1.68	2.06	2.09	2.19	3.26
	Wheat Midds	1.86	2.25	2.30	2.03	3.66
Amino acids	High-protein	2.01	2.64	2.67	2.29	3.72
	Low-protein	2.16	2.57	2.72	2.25	3.61
Mean of column		2.13	2.63	2.70	2.31	3.76
Ratio to present mean			1.24	1.27	1.08	1.76

<sup>a</sup>The energy systems considered are:

Present: Values measured in the present project.

INRA-calc: Mean of the values predicted by Equations 2 and 4 of the French INRA Net Energy system.

CVB: From the prediction equation of the Dutch CVB Net Energy system.

PPE: From the Danish Potential Physiological Energy system.

ME(NRC): Calculated from tabular ME values from NRC.



**Table 2. Net energy values (Mcal/kg dry matter) of diets measured in the present project and predicted values of those diets from other energy systems: Finishing pigs<sup>a</sup>**

Experiment	Diet	Present	INRA- calc	CVB	PPE	ME (NRC)
Corn	Low-fat basal	2.36	2.70	2.78	2.40	3.65
	Low-fat corn	2.48	2.70	2.76	2.37	3.70
	High-fat basal	2.64	3.04	3.13	2.57	4.02
	High-fat corn	2.62	3.02	3.04	2.55	3.97
Soybean meal	Basal	2.38	2.74	2.76	2.52	3.74
	Regular	2.30	2.67	2.73	2.34	3.75
	Low-oligosaccharide	2.41	2.60	2.74	2.34	3.75
Fat	Basal	2.31	2.69	2.65	2.19	3.71
	Soy oil, 5%	2.53	2.93	2.99	2.51	3.94
	Soy oil, 10%	2.59	3.23	3.24	2.80	4.18
	Choice white grease	2.68	3.29	3.42	2.97	4.13
Fiber	Basal	2.39	2.66	2.72	2.43	3.81
	Soy Hulls	1.94	2.15	2.18	1.84	3.29
	Wheat Midds	1.96	2.27	2.27	2.15	3.68
Amino acids	High-protein	2.22	2.85	2.87	2.37	3.74
	Low-protein	1.94	2.78	2.83	2.41	3.65
Mean of column		2.36	2.77	2.82	2.42	3.79
Ratio to present mean			1.17	1.20	1.03	1.61

<sup>a</sup>The energy systems considered are:

Present: Values measured in the present project.

INRA-calc: Mean of the values predicted by Equations 2 and 4 of the French INRA Net Energy system.

CVB: From the prediction equation of the Dutch CVB Net Energy system.

PPE: From the Danish Potential Physiological Energy system.

ME(NRC): Calculated from tabular ME values from NRC.

**Table 3. Net energy values (Mcal/kg dry matter) of ingredients measured in the present project and predicted values of those ingredients from other energy systems: Growing pigs<sup>a</sup>**

Experiment	Ingredient	Present	INRA- calc	INRA- tab	CVB	PPE	ME(NRC)
Corn	Corn in low fat	2.06	2.62	3.07	2.71	2.54	3.84
	Corn in high fat	1.92	2.60	3.07	2.66	2.54	3.84
SBM	Regular	1.63	2.44	2.20	2.52	1.99	3.76
	Low-oligosaccharide	1.99	2.39	2.20	2.52	1.93	3.76
Lipids	Soy oil, 5%	4.44	8.02	7.91	9.12	6.79	8.40
	Soy oil, 10%	4.66	7.36	7.91	8.81	6.79	8.40
	Choice white grease	5.83	7.56	7.91	8.18	6.72	7.96
Fiber	Soy Hulls	0.30	1.42	1.12	1.52	0.33	2.09
	Wheat Midds	0.93	2.02	2.09	2.15	1.57	3.40
Mean of column		2.64	4.05	4.17	4.47	3.47	5.05
Ratio to present mean			1.53	1.58	1.69	1.31	1.91

<sup>a</sup>The energy systems considered are:

Present: Values measured in the present project.

INRA-calc: Mean of the values predicted by Equations 2 and 4 of the French INRA Net Energy system.

INRA-tab: Ingredient NE value from INRA tables.

CVB: From the prediction equation of the Dutch CVB Net Energy system.

PPE: From the Danish Potential Physiological Energy system.

ME(NRC): Calculated from tabular ME values from NRC.

**Table 4. Net energy values (Mcal/kg dry matter) of ingredients measured in the present project and predicted values of those ingredients from other energy systems: Finishing pigs<sup>a</sup>**

	Ingredient	Present	INRA- calc	INRA- tab	CVB	PPE	ME(NRC)
Corn	Corn in low fat	2.87	2.70	3.07	2.69	2.53	3.84
	Corn in high fat	2.67	3.00	3.07	2.82	2.53	3.84
SBM	Regular	2.15	2.53	2.20	2.62	1.99	3.76
	Low-oligosaccharide	2.55	2.29	2.20	2.45	1.93	3.76
Lipids	Soy oil, 5%	5.40	6.25	7.91	6.96	6.79	8.40
	Soy oil, 10%	4.49	7.08	7.91	7.46	6.79	8.40
	Choice white grease	5.65	7.78	7.91	8.47	6.72	7.96
Fiber	Soy Hulls	0.88	0.93	1.12	1.06	0.33	2.09
	Wheat Midds	1.01	1.38	2.09	1.61	1.57	3.40
	Mean	3.08	3.77	4.17	4.01	3.46	5.05
	Rel. to Present		1.23	1.35	1.30	1.13	1.64

<sup>a</sup>The energy systems considered are:

Present: Values measured in the present project.

INRA-calc: Mean of the values predicted by Equations 2 and 4 of the French INRA Net Energy system.

INRA-tab: Ingredient NE value from INRA tables.

CVB: From the prediction equation of the Dutch CVB Net Energy system.

PPE: From the Danish Potential Physiological Energy system.

ME(NRC): Calculated from tabular ME values from NRC.

**Table 5. Ratios of NE values of various ingredients to that of corn: Growing pigs<sup>a</sup>**

Experiment	Ingredient	Present	INRA- calc	INRA- tab	CVB	PPE	ME(NRC)
SBM	Regular	0.82	0.93	0.72	0.94	0.78	0.98
	Low-oligosaccharide	1.00	0.92	0.72	0.94	0.76	0.98
Lipids	Soy oil, 5%	2.24	3.07	2.58	3.40	2.67	2.19
	Soy oil, 10%	2.35	2.82	2.58	3.28	2.67	2.19
	Choice white grease	2.93	2.89	2.58	3.05	2.64	2.07
Fiber	Soy Hulls	0.15	0.54	0.37	0.56	0.13	0.54
	Wheat Midds	0.47	0.77	0.68	0.80	0.62	0.88

<sup>a</sup>The energy systems considered are:

Present: Values measured in the present project.

INRA-calc: Mean of the values predicted by Equations 2 and 4 of the French INRA Net Energy system.

INRA-tab: Ingredient NE value from INRA tables.

CVB: From the prediction equation of the Dutch CVB Net Energy system.

PPE: From the Danish Potential Physiological Energy system.

ME(NRC): Calculated from tabular ME values from NRC.

**Table 6. Ratios of NE values of various ingredients to that of corn: Finishing pigs<sup>a</sup>**

Experiment	Ingredient	Present	INRA- calc	INRA- tab	CVB	PPE	ME(NRC)
SBM	Regular	0.78	0.89	0.72	0.95	0.79	0.98
	Low-oligosaccharide	0.92	0.80	0.72	0.89	0.76	0.98
Lipids	Soy oil, 5%	1.95	2.19	2.58	2.53	2.69	2.19
	Soy oil, 10%	1.62	2.49	2.58	2.71	2.69	2.19
	Choice white grease	2.04	2.73	2.58	3.08	2.66	2.07
Fiber	Soy Hulls	0.32	0.33	0.37	0.39	0.13	0.54
	Wheat Midds	0.37	0.48	0.68	0.58	0.62	0.88

<sup>a</sup>The energy systems considered are:

Present: Values measured in the present project.

INRA-calc: Mean of the values predicted by Equations 2 and 4 of the French INRA Net Energy system.

INRA-tab: Ingredient NE value from INRA tables.

CVB: From the prediction equation of the Dutch CVB Net Energy system.

PPE: From the Danish Potential Physiological Energy system.

ME(NRC): Calculated from tabular ME values from NRC.

**Table 7. Comparison of NE values (Mcal/kg dry matter) of ingredients for growing and finishing pigs measured in the present project**

Experiment	Ingredient	Grower	Finisher	Difference <sup>a</sup>
Corn	Corn in low fat	2.06	2.87	0.81
	Corn in high fat	1.92	2.67	0.75
SBM	Regular	1.63	2.15	0.52
	Low-oligosaccharide	1.99	2.55	0.56
Lipids	Soy oil, 5%	4.44	5.40	0.96
	Soy oil, 10%	4.66	4.49	-0.17
	Choice white grease	5.83	5.65	-0.17
Fiber	Soy Hulls	0.30	0.88	0.59
	Wheat Midds	0.93	1.01	0.09

<sup>a</sup>Finisher minus grower

# A Perspective on Changes in the Feed Industry in the Next Ten Years

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## Introduction

A now famous Harvard Business School study reviewed the multi-national company, Nestlé (Goldberg and Herman, 2005). This highly successful company is known for product successes such as Nescafé and Kit-Kat. In this case study, the company's CEO, Mr. Peter Brabeck describes where he envisions the 67 billion dollar company going, and how science could help the company get there by developing new strategies and products needed to face an ever-changing world. He asked the question: What should we eat?

In the study, Brabeck declares that nutrition in the future will focus on wellness driven by scientific research and state-of-the-art disciplines, such as nutrigenomics. He explains nutrigenomics as the relationship between diet, gene function, and health, claiming that by understanding nutrigenomics we can produce revolutionary product ideas and redefine what we eat. Why then should we follow suit, and can nutrigenomics truly help us develop new nutritional strategies? Why should a commercial animal feed ingredient company be interested in this approach? Where can you learn about nutrigenomics, and has it supplanted or replaced much of our traditional way of thinking about nutrition?

Since 2009, the animal production industry has faced harsh and cold realities. These are the realities that we need to confront: the reality of ever-vigilant scrutiny by regulators, the reality of a litigious market place, the reality of the demand for total traceability, the reality that 40 million Americans are receiving food stamps, and that our corn crops for 2009- 2010 have never been in a worse state (Robertson, 2009). For example, in Korea, a major importer of grains

from the U.S., the values of breakage and cracked grains are now approaching 20 percent; whereas in the past, they had values of only two to four percent. Furthermore, a disproportionate amount of these grains contain mycotoxins, and when scrutinized, many of our other raw materials are considered to be heavily contaminated. A case in point, in our own factories where we produce a range of minerals called Bioplexes; we routinely reject 33 percent of all minerals considered for purchase because of their contamination with dioxins, polychlorinated biphenols (PCB's), lead, and cadmium. Indeed it is virtually impossible to obtain a source of sulfate which has not been contaminated with PCB's, presumably because so much of the copper sulfate comes from the recycling of copper wires where the plastic and rubber coating has been burnt off, leaving traces of these highly toxic compounds. The feed industry must face these realities.

## Increasing supply while maintaining consumer confidence:

Along with confronting harsh realities, our industry must also meet the demands of our growing world. We need to double meat production, particularly in developing countries, by 2015. As reported recently by the United Nations Food and Agriculture Organization (FAO, 2009), production of the global livestock sector must go from the current 228 million tons of beef to 500 million tons during the next 40 years. The rapid increase in human population over the 20th century has become a new driving force for the high demand of meat products.

Are we ready for a world of nine billion people? Can new “omic” technologies help? Robert Asher once said that we have to measure success not by what we have done, but by what we could do. With “omics” we can do a lot, but we have to embrace these innovations and embrace them quickly. As you look at these challenges: food safety, food-borne toxins, mineral contamination, improvement in animal performance and fertility, it is clear that we have urgent decisions to make and more importantly, actions to take if consumer confidence in agriculture and food production is to be maintained.

## Food safety and traceability

It is clear that many regulatory issues in the future will focus on the safety of our food sources. In a recent article entitled, “A View of Health Risk Associated with Contaminated Minerals”, by David Byrne, former EU commissioner for health and food safety, it is suggested that we paraphrase the late John F. Kennedy, “Ask not what regulators can do for you, but what you can do for regulators”. Presenting this ideal, Mr. Byrne encourages us to realize that if we do not do something to address food traceability, then we will be regulated to do so (Byrne, 2010).

Times are changing and regulatory bodies are adopting a policy of “zero-tolerance” towards food and feed contaminants. In earlier days, it was sufficient to just say whether a contaminant was or was not present. Our contaminant-detection techniques have improved; we have gone from qualitative test kits to semi-quantitative test kits, from ELISA analysis to GC analysis, and from HPLC all the way up now to Mass Spectrometry (MS). With each improvement in analytical technology comes increased detection. We have gone from parts-per-million (ppm) to parts-per-quadrillion (ppq) in only 10 to 15 short years. With this quantum leap in analytical sensitivity, un-wanted substances are more easily detected, and regardless of level, the meat, produce, or ingredient containing the substance must not be introduced into the food chain. If this theory is exercised, then there will come a point when no source of food will technically be safe to eat because of course, contaminants are ubiquitous, albeit in miniscule quantities in most instances. What is the appropriate and biologically relevant level of a given contaminant needed to justify the banning of the food containing it? Genomics and microarray analysis can provide that very answer by revealing the

level a contaminant can elicit biologically relevant responses. We can now rapidly establish using this new science, whether or not a contaminant promotes cancer, impairs fertility or immune response etc., and thus determine if we need to be fearful of it.

The microarrays commonly used in nutrigenomics are basically a laboratory on a chip that can rapidly measure animal responses and define biological hazards. An added advantage is that we are vastly reducing or even bypassing the use of test animals with this technology. Using very few animals or with using animal cell cultures, known and unknown compounds can be tested to see what gene expression profile changes they induce, what genes they up-regulate or down-regulate. It is important to remember that every class of compound gives a unique gene expression fingerprint. Today, using gene expression databases generated from libraries of chemical compounds, we can rapidly cross-match unidentified contaminants through their gene expression profile, or “fingerprint”; thus identifying and assigning toxic threshold concentrations to them.

How can this be useful to us as we evaluate food supply challenges? Alltech currently produces chelated Bioplex minerals in five different facilities around the world. These facilities work 24 hours a day, seven days a week, and require special attention since more than 33 percent of the minerals we receive as raw materials are rejected because of contamination with dioxins or heavy metals. This requires special analytical techniques and considerable expense to ensure the delivery of safe products at the end of the production process. However, we see on the horizon much more efficient testing procedures using, for example, the gene chip and genomic approaches that will more rapidly and more effectively evaluate the risks associated with incoming raw materials. Such biologically-based assays will provide a new level of security for producers and help bolster consumer confidence in a world still reeling from recent food safety scares.

Mycotoxin contamination in our grains is a major threat to both the food industry and the food chain. Mycotoxins are often called, “hidden killers” and have many potential health implications when they enter the food chain. Using another “omic” technology referred to as glycomics, we are able to identify the structure of mycotoxins and build solutions around them to fundamentally eliminate their negative impact. When major grain distributors begin to recognize the problems associated with

mycotoxins like distiller's dried grains for example, they begin to issue warnings of their use in animal feeds; we need to do something. Major meat producers talk about the poor quality of the U.S. corn they are forced to use, and its inability to support the optimal weight gain of cattle, hogs, and poultry; we need to be concerned.

Over the course of the last decade, several Ph.D and Master's students have dedicated their theses to this problem. In the future, we will be able to use genomic approaches to detect in advance problems associated with the existence of mycotoxins by evaluating potential problems associated with a given feed. For example, we found that when looking at endophyte-infected feed, several hundred genes were transcriptionally altered in target animal tissues. Using this data, we can search for key biomarker genes that will support rapid-screening techniques; a research-based approach to risk management. Can we or can we not use that grain? In the next few years we will have the ability to rapidly remove individual ingredients with any perceived risk, we will develop health programs to cope with potential problems this ingredient may induce, and we will mitigate the problems that have been identified.

Are we getting there? Are we making progress? Can we produce revolutionary new product ideas? The agriculture of the future needs to focus on animal health, animal performance, and animal fertility. Nutrigenomics allows us to develop new products while at the same time explaining on a molecular level how the existing products work. For example, in dairy cows, numerous trials had demonstrated increases in milk production associated with supplementation of organic selenium, Sel-Plex® (Alltech, Nicholasville, KY) in the diet. On average, fat corrected milk production was 1.9 kilos greater per cow per day when that animal was supplemented with Sel-Plex® as opposed to inorganic sodium selenite (Silvestre et al., 2007)). The question is why? While many theories and concepts were put forward to explain the observed increase, it was not until we started using nutrigenomics that we could really explain what was happening at the molecular and cellular level. Milk production is driven by the metabolic pathways associated with energy production in the form of ATP occurring in the mitochondria. When we looked at the genes associated with energy metabolism, particularly the genes involved in oxidative phosphorylation, we saw that sodium selenite had limited effect,

but supplementation with Sel-Plex® significantly up-regulated many of the genes associated with the production of ATP; the energy currency of the cell. Increased ATP synthesis indicates more efficient diet utilization leaving more energy for milk production as a result.

Another area where the relatively new science of nutrigenomics may be of benefit is in the improvement of reproductive potential. Fertility, of course, is a problem associated not just with animals, but is a rising problem in human populations also. Selenium deficiency has long been associated with decreased fertility rates, particularly in males. For example, Edens (2002) observed severe abnormalities in the spermatozoa of roosters maintained on a selenium-deficient diet (Table 1). These were alleviated to a large extent by the addition of sodium selenite to the diet but were almost completely reversed when Sel-Plex® was used as the selenium source. What is the underlying mechanism for improved sperm quality in males in response to selenium? In our own studies using broiler breeder hens and roosters maintained on selenium-deficient, Sel-Plex®-supplemented, and sodium selenite-supplemented diets, respectively, microarray analysis quickly provided the explanation for the observed enhancement of sperm quality. We noted significant up-regulation of the gene encoding selenoprotein P1 (SEPP-1) in response to Sel-Plex® supplementation. This selenoprotein provides selenium to glutathione peroxidase 4 (GSHPx4), the corresponding gene for which was also up-regulated by Sel-Plex®, but not by sodium selenite (Figure 1). In mature sperm, GSHPx4 forms a keratin-like structure in the mid-piece of sperm, and thus is essential for mid-piece architecture and integrity. Thus, the molecular mechanism of action of Sel-Plex® in improving sperm quality seems to be centered on improved structural characteristics via up-regulation of both SEPP-1 and GSHPx-4.

We also observed significant improvements in breeder hen performance, as evidenced by increased settable egg and chick production. Again, our "lab on a chip" approach provided a molecular explanation for previous inexplicable enhancements in female fertility in response to supplementation with certain selenium forms. For example, we noted potent up-regulation of genes encoding important growth and transcription factors in response to Sel-Plex® but not to selenite. Likewise, the key genes involved in the follicle stimulating hormone (FSH) cascade were

uniquely up-regulated in Sel-Plex®-supplemented hens. Results generated in reproductive tissues, such as oviduct, are not necessarily restricted to the species in which they are observed. Indeed, in the particular case of hen oviduct, observations may be extrapolated to a whole range of species and it is used, for example, as a model tissue for human fertility studies (Dougherty and Sanders, 2005).

So what does this mean? It means that the agriculture industry is moving forward at a new speed and urgency allowing us to rapidly develop and test new ingredients for the enhancement of production, fertility, of course in product quality.

### **Product quality: Finding alternative antioxidants**

To demonstrate the power and speed of this new technology, we tasked our own nutrigenomics team with finding alternatives to expensive feed ingredients, such as the use of antioxidant and vitamin E. Antioxidants are required for optimum growth, immunity and reproduction. Historically, nutritionists have added vitamin E at 5 to 10 times NRC requirements to meet the perceived need and maintain product quality. Supplies of vitamin E are limited, and historically, suppliers have been known to adjust or manipulate prices, holding back production. Through our understanding of the biochemical basis for antioxidant action and effect, coupled with explorations of gene chip technology, we have been able to closely mimic the global transcriptional changes which vitamin E elicits in tissue such as skeletal muscle; with a revolutionary new product we have named EconomasE™. We satisfied ourselves with the finding that EconomasE™ alters gene expression profiles in a manner unidirectional to vitamin E; conducting extensive validation studies in conjunction with the University of Kentucky at their Coldstream facility. Observations arising from trials to test the in-vivo application of our in silico measurements included: higher total antioxidant capacity (TAC) in the serum of EconomasE™-supplemented versus vitamin E-supplemented birds, lower drip loss post-slaughter in EconomasE™ groups, and much better retention of meat color in EconomasE™-treated versus vitamin E-treated birds. In practical terms at the retail level, this translates into a product of much greater “eye-appeal” to the consumer, much longer shelf-life, and enhanced retention of nutrients. Our

findings are not restricted to chicken meat; initial trials in beef animals have demonstrated similar reduced levels of oxidative stress, reduced levels of peroxidative damage, and enhanced redness/shelf-life in EconomasE™-supplemented versus standard beef. We now have a platform for utilizing less expensive nutrients, which can be rapidly tested and validated using the nutrigenomic approach. Truly this is a revolutionary lesson and we are rewriting the book on nutrient requirements.

### **Functional Foods and Human Health**

How can this new science of nutrigenomics impact human health? In a recent issue of USA today, it was observed that in the next decade there will be two “pandemics” that will dominate the medical health area; Alzheimer’s disease (AD) and cancer. Are there any leads that we can obtain from nutrigenomics that will help in these two areas? A clue to the future is often in the past. In some pioneering work on caloric restriction and its impact on aging, Professor Richard Weindruch, at the University of Wisconsin, found that when monkeys were put on a calorie-restricted diet they aged less rapidly than monkeys receiving a normal diet (Anderson, 2009). A clear visual difference could be observed. It wasn’t however until we started looking at the gene expression patterns that we got a clue as to what might possibly be happening molecularly. It was observed that the gene expression patterns on the high calorie diet were similar to those resulting from a selenium-deficient diet. Could selenium be involved? Knowing that the most popular theory of aging mechanistic centers on cumulative damage caused by oxygen radicals, we performed studies in laboratory animals which indeed showed that we could get significantly less oxidative damage in those mice fed Sel-Plex than those not fed Sel-Plex. However, we also made the intriguing observation in the mouse brain that Sel-Plex caused the down-regulation of a number of key genes known to be involved in the development of AD in humans. With this as a lead, we decided to look at AD in more detail using nutrigenomic methodologies.

Alzheimer’s disease is one of the most devastating diseases facing the world. It is a progressive, irreversible, neurodegenerative disorder for which there is no known cure. According to the Alzheimer’s Association, AD currently affects 5 to



6 million Americans and this number is expected to rise to 16 million by 2050. The annual cost of AD care is predicted to become the single greatest drain on health care systems over the next 10 to 15 years; surpassing even that of Type-II diabetes (Alzheimer's Association, 2010). A preventative measure which could delay AD onset by even one to two years would mean six million less Americans suffering from AD, with massive economic consequences. The world's leading institute on Alzheimer's disease is the Sanders Brown Center of Aging at the University of Kentucky. Here, the late Professor William Markesbery, MD pioneered work on Alzheimer's. Professor Markesbery was a tireless worker in this area and was fascinated by some of the work that we were seeing on animals receiving Sel-Plex®. He decided to look at the impact of selenium supplementation on the production of amyloid plaques in a unique mouse model of AD. This strain of mouse contains in its brain multiple copies of human genes responsible for amyloid plaque formation. In a breakthrough paper published in *Free Radical Biology & Medicine*, he reported that he was observing fewer plaques in the brain tissue of the animals fed Sel-Plex® (Lovell et al., 2009). This breakthrough, he went on to say, should lead to the use of Sel-Plex® as a potential therapeutic agent for slowing down the development of Alzheimer's.

Any leads on cancer? Once again, prompted by observation from gene chip experiments, we initiated collaborative work with Professor Michael Toborek's group in the Department of Neurosurgery at the University of Kentucky. In his experiments, mice were raised to four months of ages on selenium deficient, selenomethionine, sodium selenite, and Sel-Plex®-supplemented diets, respectively. Following this period, each mouse was injected via its external carotid artery, with a fluorescently tagged cancer cell line and the development of the resulting brain tumors monitored via luminometry. Toborek's team found that the cancer developed at a rapid pace in all groups with the exception of the group receiving Sel-Plex®. In these animals, a small tumor developed but was held in check in a single location with no measurable growth occurring over time. Similar work has been performed using selenium-enriched milk from cows fed Sel-Plex®. Here, casein powder isolated from such milk has been found to have a very significant impact on reducing tumor load in laboratory animal cancer models, thus opening the possibility for novel functional foods with anti-cancer

potential.

Are we on the right track? We believe that we are. Nutrigenomics is now providing us with new tools for addressing nutritional issues associated with animal health and productivity. These also have great implications for human health, either by directly developing supplements for human consumption or by enriching animal produce to generate functional foods for such purposes. We now can rapidly develop products and use fewer resources such as test animals, personnel and facilities. We can find new ways of defining nutrition to meet physiological needs. Equally, we can explain real-time nutritional and physiological responses at the actual molecular level. Will the nutrition of the future, the nutrition of health and wellness, indeed be driven by nutrigenomics? We believe the answer is yes. We believe this exciting new tool, providing a molecular understanding of how common nutrients or chemicals can affect health and performance, will give us the ability to redefine nutrition through the feeding of genes.

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Table 1:

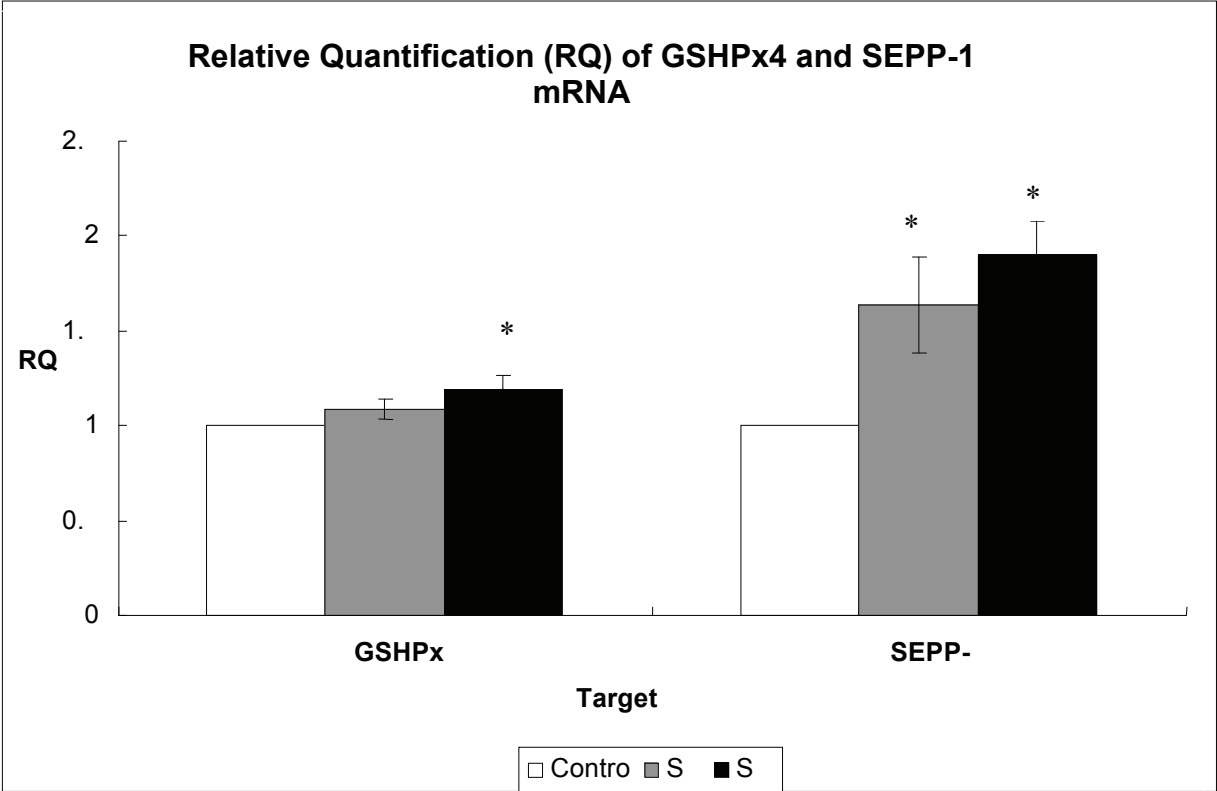
**Effect of selenium source on fertility in Breeder Hens**

Spermatozoal abnormalities (%) found in semen from roosters given feeds with either sodium selenite, Sel-Plex® or no supplemental selenium.

	<b>Basal</b>	<b>Selenite</b>	<b>Sel-Plex</b>
<b>Normal sperm</b>	57.9 <sup>c</sup>	89.4 <sup>b</sup>	98.7 <sup>a</sup>
<b>Bent midpiece</b>	18.7 <sup>a</sup>	6.2 <sup>b</sup>	0.7 <sup>c</sup>
<b>Swollen midpiece</b>	1.6 <sup>a</sup>	0.4 <sup>b</sup>	0.1 <sup>c</sup>
<b>Ruptured midpiece</b>	0.9 <sup>a</sup>	0.1 <sup>b</sup>	0.0 <sup>b</sup>
<b>Swollen head</b>	1.3 <sup>a</sup>	0.2 <sup>b</sup>	0.2 <sup>b</sup>
<b>Corkscrew head</b>	15.4 <sup>a</sup>	1.8 <sup>b</sup>	0.2 <sup>c</sup>
<b>Coiled</b>	3.2 <sup>a</sup>	0.8 <sup>b</sup>	0.0 <sup>c</sup>
<b>Fragment/Other</b>	1.0 <sup>a</sup>	1.1 <sup>a</sup>	0.1 <sup>b</sup>

\* a,b,c Means in a row differ significantly (P<0.05).

Figure 1:



\* p<0.05

# Linking our Understanding of Mammary Gland Metabolism to Sow Amino Acid Nutrition

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## Summary

Imbalances created by excesses or deficiencies of dietary AA reduce the global efficiency of dietary protein utilization by the animal by limiting milk protein synthesis and/or increasing N losses to the environment. Understanding the fate of AA is thus crucial to optimizing AA utilization for milk protein synthesis and to reducing inefficiencies. For example, is there an obligate AA oxidation? By understanding the functional role of AA metabolism, could we in the near future uncover pathways and molecular targets to significantly improve AA utilization? The authors highlight processes of mammary AA utilization in response to AA nutrition of the sow, and propose that knowledge of such processes offers impetus for refining the AA requirement for lactation.

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## Introduction

Several studies have addressed the impact of optimizing ratios between dietary lysine and other AA in lactating sow diets, including valine alone (Richert et al., 1996, 1997a), the branched-chain AA (Richert et al., 1997b; Moser et al., 2000), and threonine (Cooper et al., 2001) on litter growth. However, very little attention has been paid to the mechanisms linking dietary AA profiling to milk protein synthesis. The rate at which circulating AA are extracted by the mammary gland during lactation varies anywhere between 20 and 60% depending on the AA and its availability. We are only beginning to unveil the fate of mammary-extracted AA and the myriad of mammary AA metabolism events accompanying the milk protein synthetic process.

In the discussion below, we have attempted to summarize the current body of knowledge on mammary AA metabolism in the lactating sow and its relevance to the sow and piglet AA nutrition and requirement. We discuss the major pathways of AA utilization for growth and protein synthesis, how the mammary gland contributes to the N economy and

global N efficiency of the animal, and the metabolic pathways known to date in mammary tissue. It is well recognized that the sum of indispensable AA uptake by the lactating mammary gland exceeds that sum in the secreted milk (Trottier et al., 1997; Trottier and Guan 2000; Guan et al., 2004). Of the total 188.5 g of essential AA taken up by the sow mammary gland daily, 49 g is retained, accounting for about 25% of the total uptake (Trottier et al. 1997). Consequently, there is a substantial use of indispensable AA in mammary metabolic pathways, including oxidation, synthesis of dispensable AA, structural and functional proteins, and miscellaneous compounds, and mammary tissue remodeling.

## *Mammary Gland Growth*

During lactation, relative to the daily quantity of milk protein synthesized, there is little net mammary gland growth, despite two-fold-increase in total mammary DNA from parturition to day 21 of lactation in gilts (Kim et al., 1999). On the other hand, total RNA concentration linearly increases from parturition until day 21 of lactation,

indicating increasing cellular protein synthetic activity (Manjarin and Trottier, unpublished), and in agreement with our model-derived estimate of mammary protein turnover rate; we determined through transmembrane flux kinetics measurement of lysine, methionine and valine, that the mammary gland of a lactating sow producing 9 kg milk daily synthesizes and breaks down daily, respectively, 975 and 400 g of proteins (Guan et al., 2002), illustrating the extent of metabolism occurring within the tissue. The net protein balance derived from the protein synthesis and breakdown model is estimated at 575 g, which compares well with the calculated value of 529 g [milk protein output of 514 g + mammary protein deposition of 14.8 g, estimated from figures of Kim et al. (1999)]. Thus only 14.8 g of protein can be accounted for mammary growth, representing 2.6 and 2.8% of the net mammary protein balance, based on the derived model of 575 g or the calculated figure of 514 g protein balance per day, respectively. As it happens, there is no significant increase in DNA concentration in mammary tissue from parturition to day 21 of lactation in multiparous sows, indicating that is little net mammary gland growth in multiparous sows (Manjarin and Trottier, unpublished).

#### ***Feeding less protein for better efficiency: focus on mammary metabolism***

Dietary AA supply to the dam below the requirement level depresses milk protein yield and subsequent neonatal pig growth. Likewise, excessive dietary AA supply to the dam depresses piglet growth and does so to a greater extent than that of dietary AA deficiency (King et al., 1993; Yang et al., 2000). These changes are accompanied by what seems to be some adaptive mechanisms whereby the mammary gland modulates the extraction rates of circulating AA in response to dietary AA availability, on one hand in an attempt to meet the need of milk protein demand but on the other hand to limit the unnecessary uptake of certain AA (Guan et al., 2004). However, limiting uptake of superfluous AA is costly and inefficient; not only because of the metabolic processes associated with AA catabolism and N excretion, but because the mammary glands' ability to down regulate nutrient flow and uptake appears limited. In a recent study (Pérez laspiur et al., 2009) using a nutritional stress model, we fed

lactating sows a deficient-protein diet containing 12% CP, an adequate diet (18% CP) and an excess-protein diet. As expected, feeding a 12%-CP diet led to lower sow plasma IAA concentrations which directly limited milk casein yield and piglet growth; in contrast, feeding an excessive concentration of dietary CP (24%), despite higher IAA plasma concentrations, clearly depressed milk casein yield and piglet growth (Figure 1). There was a clear curvilinear response of piglet growth and casein yield such that under both conditions of nutritional stress, either protein deficiency or protein excess, low milk casein yield appeared to limit piglet growth. We also showed in an earlier study that lactating sows fed diets with increasing dietary protein concentrations (7.8 to 23.5% CP) respond by decreasing mammary transport of cationic (lysine and arginine) and other neutral AA (threonine) and by increasing transport of leucine and isoleucine (Guan et al., 2004) (Figure 2).

We have recently (Manjarin et al., unpublished) examined if a similar response is relevant in sows fed diets formulated to match an ideal AA profile using the NRC (1998) model. In that study, we investigated mammary gland AA extraction rate, measured using the arteriovenous difference approach, in response to different levels of dietary AA and CP concentrations fed to lactating sows. We formulated a diet containing 13.5% CP and crystalline AA to both meet requirements and match AA profile reported in NRC (1998) (labeled as "ideal") and a diet containing 17.5% CP also formulated to match NRC (1998) profile (labeled as "standard"). We also used a diet deficient in CP (9.5%) to estimate additional efficiency gain of dietary protein reduction under a scenario of AA deficiency. Voluntary feed intake and lactation weight loss did not differ between diets, and ADG of piglets from sows fed the ideal diet did not differ from that of sows fed the standard diet (Table 1). However, sows fed the ideal diet exhibited higher AA extraction rates in particular for those typically dietary limiting compared to sows fed the standard diet, indicating that the ideal-fed sows were more efficient at utilizing circulating AA. The mammary response to a dietary CP excess model supports our earlier arguments in favor of AA interactions at the basolateral aspect of the mammary cell, and the likely mechanistic basis behind ideal AA profile. Evidence for such proposal is discussed below.

### ***Evidence of interaction between cationic and branched-chain amino acids***

Several *ex vivo* and *in vivo* studies support the notion that an interaction exist between cationic and the branched-chain AA for transport across the basolateral membrane of the mammary epithelial cell. The first evidence stemmed from the work of Shennan et al. (1994) and Calvert and Shennan (1996) who demonstrated that physiological concentrations of leucine strongly inhibits valine and lysine uptake by mammary tissue explants collected from lactating rats. Later, a similar response was reported with mammary tissue explants collected from lactating sows (Jackson et al., 2000). Lysine also inhibited up to 67% valine uptake by mammary tissue explants collected from lactating sows (Hurley et al., 2000).

Although the nature of these interactions between cationic and neutral AA in the mammary gland determined on mammary tissue explants (i.e., *ex vivo*) remain to be understood, a sequence of *in vivo* studies clearly corroborate the *ex vivo* findings. Dietary over-supplementation with purified L-lysine•HCl in sow diets appears to lead to decrease in valine utilization (Richert et al., 1996, 1997). Conversely, we found that dietary over-supplementation of crystalline L-valine decreases lysine mammary trans-membrane transport in lactating sows by stimulating lysine outward movement (Guan et al., 2002). We also showed in the mammary arteriovenous difference model, and presented in the above section, that lactating sows fed diets with increasing dietary protein concentration respond by decreasing mammary transport of cationic and other neutral AA and by increasing transport of leucine and isoleucine (Guan et al., 2004) (Figure 2). Similarly, in lactating dairy cows fed dietary CP above requirement, leucine oxidation by the mammary gland increased with no increase in milk protein yield (Bequette et al., 1996). Others have also demonstrated *in vivo*, a high capacity for BCAA oxidation by the mammary gland in rats (DeSantiago et al., 1998) and sows (Richert et al., 1998). The physiological relevance of large mammary uptake capacity and oxidation BCAA demonstrated *in vivo* and *ex vivo* is unknown. As reported in the dairy cow, we demonstrated that feeding excessive quantities of CP (24%) result in a negligible increase (i.e., 1.6%) in true milk protein concentration but a significant increase in daily leucine and isoleucine (22 and 28%, respectively) uptake per suckled gland

compared to sows fed an 18%-CP diet (Guan et al., 2004). Bequette et al. (1996) suggested earlier that reducing activities, such as oxidation, not seemingly crucial for milk synthesis, might improve the efficiency of AA conversion into milk proteins.

Feeding graded levels of CP from 7.3 to 24% and measuring the relative AA output in milk to mammary AA uptake allowed the authors (Guan et al., 2004) to approximate the relative contribution of AA to the mammary retention pool and the milk pool, and as such, allocate some efficiency estimates. Leucine milk output:mammary uptake ranged from 100% in sows fed the Deficient CP diet (7.3%) to 71% in sows fed the Excess CP diet (24%), with 82% in sows fed Low (13%) and Normal (18%) diets. Similar changes in output to uptake ratios were observed for isoleucine, valine, and arginine. Conversely, AA such as threonine, phenylalanine and methionine had consistently high output to uptake ratios (close to 100%) across all dietary treatments, suggesting minimum use, if any, of these AA into oxidative pathways. Output:uptake ratios for histidine were consistently higher than 100%, indicating *in situ* secretion of histidine by mammary tissue. In addition, when expressing mammary arteriovenous difference of an individual AA as a proportion of that of total indispensable AA (Figure 3), in response to feeding a deficient-CP diet, we were able to rank the limiting order of AA; the response was the highest for lysine, followed by threonine, phenylalanine, methionine, tryptophan and arginine. The response from all three BCAA in particular that of leucine and isoleucine, indicates these AA are not limiting.

### ***Passport to the mammary world: Role of AA transporters***

The molecular events linking the identified interactions between cationic and branched-chain AA and lactation response are unknown. The intracellular availability of dietary amino acids is controlled by a coordinated activity of AA carrier proteins located in the cellular membrane and responsible for channeling AA across the cell membranes. Regulation of amino acid transport is complex because many transporters not only handle multiple AA, but also co-transport them in and out of the cells.

*Cationic amino acid transport.* Cationic amino acid transporter (CAT) proteins are part of the Na<sup>+</sup>-independent, ubiquitously expressed system y<sup>+</sup>. This system exhibits unique affinity for transport of

cationic AA, namely arginine, histidine, lysine, and ornithine. The CAT transporters are typically pH-independent and transport activity is stimulated by membrane hyperpolarization, and by the presence of neutral AA on the *trans* side of the membrane (Closs, 2002).

*Cationic and Neutral Amino Acid Shared-Transport.* Lysine can be transported via other systems, i.e., systems  $b^{0,+}$ ,  $B^{0,+}$  and  $y^+L$ . A description of the molecular structure and function of these transporters is beyond the scope of this presentation. However, noteworthy to mention, we recently reported (Manjarin et al., 2010) that genes encoding for AA transporters CAT-1, CAT-2b,  $ATB^{0,+}$ ,  $b^{0,+}AT$ ,  $y^+LAT1$  and  $y^+LAT2$  are all transcribed in the porcine mammary parenchymal tissue, with only CAT-1,  $ATB^{0,+}$  and  $y^+LAT2$  exhibiting both significant expression and association with  $\kappa$ -casein and  $\kappa$ -lactalbumin expression at the transcription level. We propose that CAT-1,  $ATB^{0,+}$  and  $y^+LAT2$  transporters are more likely to be candidate transporters responsible for the bulk of arterial/basolateral AA extraction.

#### ***Metabolic basis for the apparent mammary arginine, branched-chain amino acids and glutamine requirement***

During the past 10 years, massive work has been conducted to profile the enzymatic machinery of sow mammary tissue, and the data has provided critical enlightenment to the arteriovenous difference data. Firstly, a significant proportion of the 46 g of apparently retained AA by the mammary gland during lactation can be traced to metabolic pathways, as illustrated in Figure 4. Based on the estimated indispensable AA retention of 46 g per day, and the net mammary tissue indispensable AA accretion of 14.8g, the estimate indispensable AA used in metabolic-related events amount to approximately 68% of the retained AA. These figures do not include the use of dispensable AA retention and metabolism, but as discussed below, glutamine, glutamate, aspartate and alanine represent a crucial aspect of mammary metabolism. Whether these pathways represent obligate losses, futile cycles or are co-regulated with global milk synthetic processes remains unknown. Nonetheless, mapping the entire amino acid metabolic processes may allow in the future targeting genes of interest and increase funneling of AA utilization into products of nutritional values for the nursing piglet, thereby

increasing the efficiency of dietary amino acid utilization.

*Arginine.* Several studies have shown that arginine is catabolized in lactating porcine mammary tissue to form proline, ornithine and urea via the arginase pathway, and small amounts of polyamines and NO via the arginase and NOS pathways (O'Quinn et al., 2002). There are two different arginases in the lactating porcine mammary tissue: arginase I (a cytosolic enzyme) and arginase II (a mitochondrial enzyme). Both enzymes cleave arginine to yield urea and ornithine. The ornithine produced in the cytosol can be either utilized for polyamine synthesis by ornithine decarboxylase (ODC) and spermidine synthase (SP; Wu and Morris, 1998), or can be transported into the mitochondria and converted to  $\Delta^1$ -L-pyrroline-5-carboxylate by the enzyme ornithine aminotransferase (OAT). Then  $\Delta^1$ -L-pyrroline-5-carboxylate is either converted to glutamate by the enzyme  $\Delta^1$ -L-pyrroline-5-carboxylate deshydrogenase (P5CD), or exported to the cytosol and converted to proline by the enzyme  $\Delta^1$ -L-pyrroline-5-carboxylate reductase (P5CR). The activity of P5CR is 56-fold greater than that of P5CD in lactating porcine mammary tissue, thus favoring the conversion of arginine-derived P5C into proline rather than into glutamate or glutamine. Moreover, porcine mammary gland lacks the enzyme  $\Delta^1$ -L-Pyrroline-5-Carboxylate synthase, and therefore proline cannot be synthesized from glutamine or glutamate by this tissue (O'Quinn et al., 2002). These notions corroborate the high arteriovenous differences of arginine and high milk proline concentrations, and likelihood that mammary arginine uptake is an essential coordinated process. Arginine is also the substrate for nitric oxide (NO) synthesis, but it is estimated to be quantitatively a minor pathway for arginine degradation in lactating mammary gland (O'Quinn et al., 2002). Nitric oxide is produced from arginine and molecular oxygen in a reaction catalyzed by the enzyme NO synthase. Nitric oxide rapidly diffuses into the tissue and regulates blood flow (Meininger and Wu, 2002; Kim and Wu, 2009) and the availability of AA to their target mammary cells. The importance of polyamines from arginine is noteworthy given the proliferative nature of the mammary tissue; however, the extent of mammary tissue proliferation during lactation is unclear, and would appear to be relevance in gilts rather than mature sows. Moreover, because polyamines can be transported by mammary cells,

de novo mammary synthesis from arginine may represent an alternative pathway.

*Branched amino acids (BCAA)*. Uptake of BCAA leucine, valine and isoleucine by porcine mammary gland (76g/d on d 13-20 of lactation) is strikingly greater than their secretion in milk protein. The lactating porcine mammary gland catabolizes approximately 30 g of BCAA per day. Several studies indicate that BCAA catabolism in mammary cells resemble catabolism of BCAA in other organs, involving 2 initial enzymatic steps (Li et al., 2009). The first step is the transamination of leucine, isoleucine and valine by the enzyme **branched-chain amino** transferase (BCAT). Li et al. (2009) reported the presence of both mammalian BCAT isozymes in mammary tissue, i.e., the mitochondrial and cytosolic isoforms. Therefore, transamination of BCAA in porcine mammary gland likely occurs in the mitochondria and the cytoplasm of mammary cells. In the transamination reaction, the  $\alpha$ -amino group of leucine, isoleucine and valine is transferred to the Krebs intermediate  $\alpha$ -ketoglutarate to form glutamate, leaving behind the corresponding  $\alpha$ -keto acids (BCKA) ( $\alpha$ -ketoisocaproate,  $\alpha$ -keto- $\beta$ -methylvalerate and  $\alpha$ -ketoisovalerate, respectively). The **branched-chain**  $\alpha$ -keto acid dehydrogenase complex (BCKD) then catalyzes oxidative decarboxylation of all three  $\alpha$ -keto acids producing the acyl-CoA derivatives. The **branched-chain**  $\alpha$ -keto acid dehydrogenase (BCKD) is a multienzyme complex located on the inner surface of the mitochondrial membrane. Therefore, if transamination of BCAA occurs in the cytoplasm by the cytosolic isoform of BCAT, the  $\alpha$ -keto acids (BCKA) produced may need to be transported to the mitochondria to complete oxidation. The next step in the oxidation of BCAA is oxidation of the acyl-CoA, catalyzed by 2 different dehydrogenases. After this step, the individual BCAA catabolic pathways diverge, producing acetyl-CoA (leucine and isoleucine) and succinyl-CoA (valine and isoleucine) that are finally incorporated into the Krebs cycle (Nelson and Cox, 2008).

*Glutamate/glutamine and aspartate/asparagine*. Glutamate/glutamine and aspartate/asparagine may have significant nutritional importance, as they are the most abundant free and protein-bound AA in sow milk at peak of lactation (Wu and Knabe, 1994). Glutamate and glutamine have the highest extraction rate by the mammary gland during lactation, whereas the extraction of aspartate/asparagine is lower than

their output in milk, suggesting their net synthesis by the mammary cells (Trottier et al., 1997). Li et al. (2009) showed that most milk aspartate is derived from transamination of glutamate, a reaction catalyzed by the enzyme glutamate oxalacetate transaminase (GOT). Alternatively, glutamate can be converted into glutamine by the cytosolic enzyme glutamine synthetase (GS). Interestingly, although the activity of GOT is reported to be higher than the activity of GS, glutamine synthesis is higher than aspartate synthesis in porcine mammary tissue (Li et al., 2009). Finally, glutamate can be transaminated with pyruvate by the enzyme glutamate pyruvate transaminase (GPT) to form alanine and  $\alpha$ -ketoglutarate. However, glutamate synthesis predominates over alanine synthesis, suggesting that the transamination reaction moves towards the formation of glutamate in the lactating sow (Li et al., 2009).\_

## Conclusion

We have highlighted the major pathways of AA metabolism in the sow mammary gland during lactation and provided some quantitative estimates. Although AA metabolism represents an important contribution to the global process of milk synthesis, it is important to recognize that leucine metabolism may not be an essential contributor and may reduce the utilization of limiting indispensable AA. In contrast, the large mammary uptake of arginine appears to be a regulated and essential process. Mammary AA extraction efficiency is improved with reduction in dietary CP intake and thus contributes to the efficiency of AA utilization at the whole animal level.

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Table 1. Effect of feeding diets containing deficient, ideal and standard concentrations of CP and digestible amino acids on sow voluntary feed intake, weight loss, and average piglet daily gain <sup>1</sup>

Item	Deficient	Ideal	Standard
Feed intake (kg/d)	4.46 ± 0.27	4.83 ± 0.27	4.47 ± 0.29
Sow weight loss (kg/d)	1.22 ± 0.27	0.95 ± 0.27	1.46 ± 0.30
ADG (g/d)	218.2 ± 11.2 <sup>b</sup>	279.6 ± 11.5 <sup>a</sup>	252.0 ± 13.3

<sup>1</sup> Values are least squares means ± SEM.

Least squares means with different letters differ at  $P < 0.05$ .

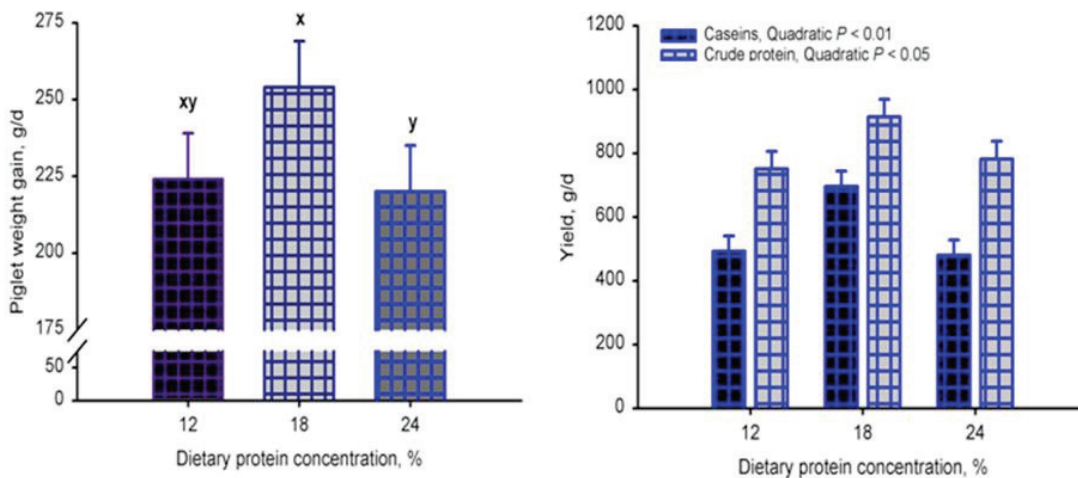


Figure 1. Effect of increasing dietary protein concentrations in lactation sow diets on piglet ADG (left) and milk caseins and true protein concentration (right). Bars are least squares means and SEM. Bars with different letters differ at  $P < 0.05$ . Data obtained from Pérez Laspiur et al. (2009).

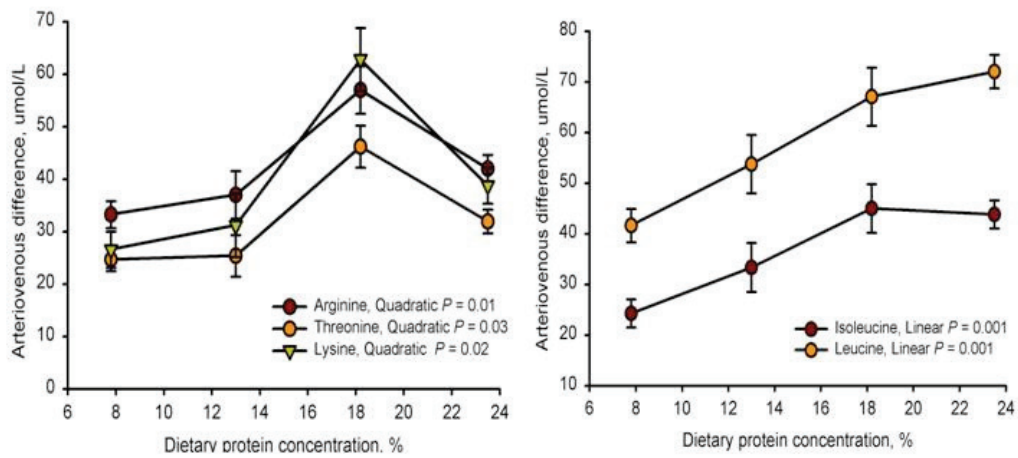


Figure 2. Mammary arteriovenous concentration difference of arginine, lysine and threonine (left) and of leucine and isoleucine (right) in sows fed increasing dietary CP concentrations (7.8 to 23.5%). Data obtained from Guan et al. (2004).

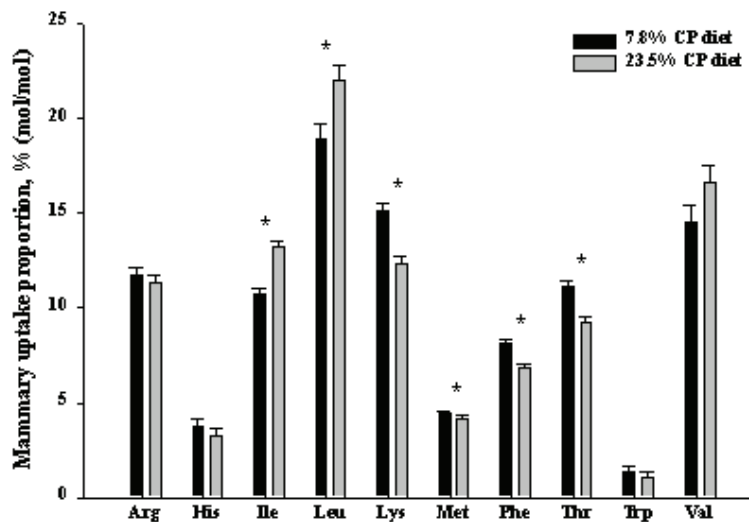


Figure 3. Effect of dietary protein concentrations in lactating sow diets on mammary arteriovenous difference of individual indispensable AA relative to the total arteriovenous difference of indispensable AA. Mammary uptake proportions (% mol/mol) is defined as  $100 \times$  mammary A-V difference of an individual IAA/ mammary A-V difference of the total IAA. Least squares means with \* differ ( $P < .05$ ) between diets. Data obtained from Guan et al. (2004).

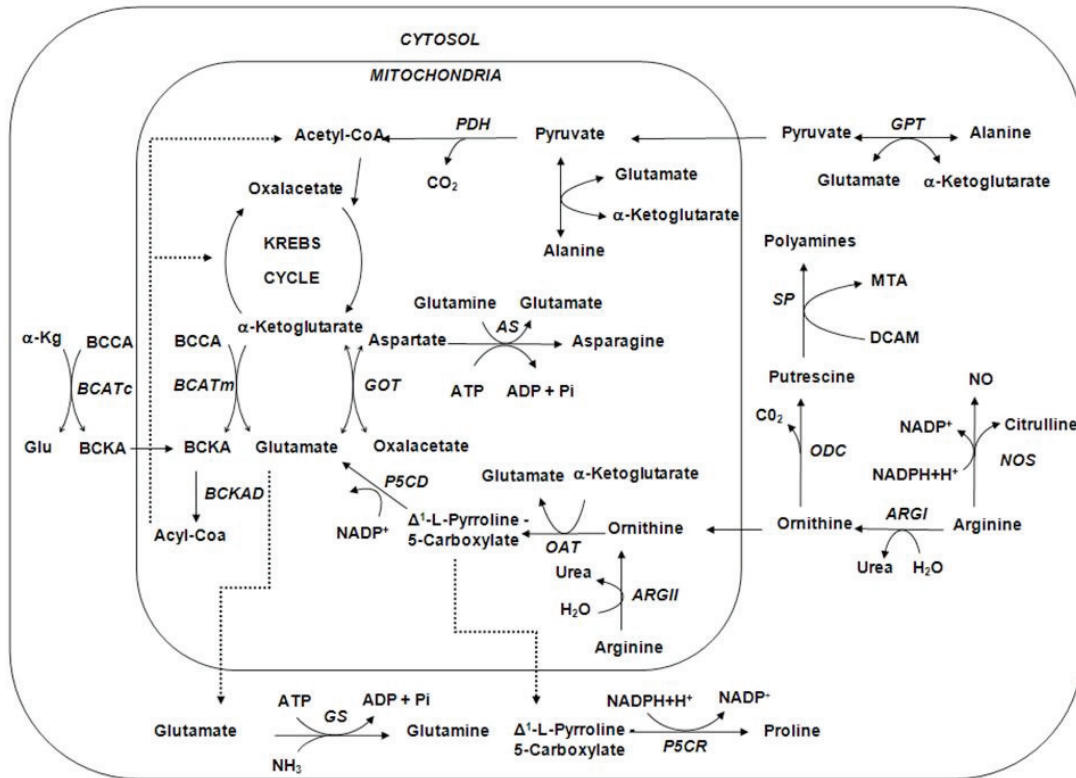


Figure 4. Overall amino acid metabolism in the mammary cell. See text for description and proper citations. Figure by Trottier and Manjarin (2010), based on the data of Wu and Morris (1998), O'Quinn et al. (2002), Nelson and Cox (2008) and Li et al. (2009).













