

# Ontario Swine Research Review 2009

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## The Crop & Energy Price Linkage: Implications for Swine Production

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### Biofuel Levels Stimulated by Policy

Primary motivation for support was:

Increase farm income

Reduce GHGs

### Reduce reliance on imported oil (US)

If all US corn and soybean crop devoted to biofuels, it would offset the US demand of gasoline by 12%

Types of programs:

Blend subsidies, import tariffs and mandates

i.e. US Renewable Fuel Standard (RFS) - Energy Independence & Security Act will increase US production 4 fold.

### Determinants of Corn Price

Corn used primarily for livestock feed, food ingredient, ethanol

Each user of corn has a maximum bid price before they switch to an alternative or shut-down.

Corn price is set by user with the lowest maximum bid price.

Ethanol is now the marginal user of corn.

### Biofuel Industry is Here to Stay!

Biofuel policy and high oil prices have established a biofuel industry

There is now a linkage between energy prices and corn prices.

a) Oil price > gas price

b) gas and ethanol are substitutes

Mandates put a floor on corn prices

Higher energy prices will pull up corn prices

Higher energy prices will not push up crop prices

### Oil, Ethanol Policy and Corn

Price of Oil (\$/barrel)	Price of Corn (\$/tonne)	
	Without Policies	With Policies
\$40	\$115	\$145
\$100	\$140	\$160

Source FAPRI, 2009

On average, corn prices 15-25% higher due to biofuel policies

### Summary

Corn price is now intricately linked to energy prices

Biofuel policy provides a new floor to corn price

While corn and soy prices can be pulled up by energy prices the reverse is not true

Farmer cannot pass on higher energy prices

# Effects of Low-CP Feeding on Growth, Nutrient Utilization and Manure Odor in Weanling Pigs

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## BACKGROUND

Dietary protein not only provides essential nutrients for body growth, but excessive dietary protein intake also leads to poor efficiency of N utilization and manure odor excretion and its impact on the nearby community.

## OBJECTIVES

The objectives of this study were to examine effects of low-protein feeding on growth performance, efficiency of dietary N and P utilization, carcass fat content and fecal total volatile sulfides in weanling pigs fed corn and SBM-based diets.

## METHODOLOGY

The study was conducted with 36 Yorkshire weanling barrows of average initial and final BW of 9.6 and 12.9 kg and the pigs were fed six diets according to a completely randomized block design. The six diets were corn and SBM-based, including diets 1-4 with graded declining levels of total CP (diet 1, control, 18.5; diet 2, 17.5; diet 3, 16.5; and diet 4, 15.5 CP%) plus diet 5 (15.1% CP+5% cellulose), and diet 6 (15.1% CP+extra crystalline L-Leu). Chromic oxide (0.30%) was included as a digestibility marker. Each experimental period consisted of 15 d with 10-d adaptation and 5-d collection of representative fecal and urinary samples.

## RESULTS

Reduction in CP content from 18.5 to 15.5% did not have effects ( $P>0.05$ ) on performance endpoints. A reduction in the net energy content of the low-CP diet with 5% cellulose (diet 5) did not affect ( $P>0.05$ ) the growth performance endpoints. While 5% cellulose supplementation (diet 5) reduced total fecal sulfide excretion, it also decreased ( $P<0.05$ ) the whole tract dry matter digestibility. Fortification of the low-CP diet with extra crystalline Leu (diet 6) did not affect ( $P>0.05$ ) the growth performance endpoints. The reduction of dietary CP content by 3% did not affect ( $P>0.05$ ) carcass fat content. Decreases in dietary CP levels had a positive linear effect ( $P<0.05$ ) on efficiency of N utilization with optimal responses observed in diets 3 and 4.

## BENEFITS TO SWINE INDUSTRY

A reduction in dietary CP content of 3% of the NRC (1998) recommended level through supplementation of crystalline limiting essential AA can significantly improve efficiency of N utilization and reduce manure N excretion for up to 20% without negatively affecting performance, carcass fat content, manure sulfide and P excretions in weanling pigs fed corn and SBM-based diets.

**ACKNOWLEDGEMENTS:** Supported by Ontario Pork and OMAFRA-University of Guelph Partnership Program.

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**ACKNOWLEDGEMENTS:** Supported by Ontario Pork and OMAFRA-University of Guelph Partnership Program.

# CEREAL PHOSPHORUS DIGESTION BY THE CASSIE LINE OF ENVIROPIG™ AND YORKSHIRE FINISHER BARROWS FED A DIET CONTAINING SUPPLEMENTAL PHYTASE

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## **Background:**

Genetically engineered pigs trademarked Enviropig™ with the capability to synthesize phytase in the salivary glands are able to use cereal grains as the sole source of phosphorus for balanced growth and development. This study was undertaken to compare the efficiency of phosphorus utilization by the Cassie line of Enviropig™ finisher barrows fed a diet lacking supplemental phosphorus with that of conventional Yorkshire finisher barrows fed the same diet, except containing added phytase.

## **Methodology:**

A feeding trial was conducted with 10 Cassie finisher barrows and 10 age matched conventional Yorkshire barrows. The trial was conducted in two parts each with 5 Cassie and 5 Yorkshire pigs in metabolic crates. Pigs were fed a diet containing 46% corn, 9.4% soybean meal, 31% barley and 10% wheat as the main constituents. The Yorkshire diet contained 750 International Units of phytase per kg of feed. The pigs were fed at 5% of body weight and water ad libitum. Water and feed consumption, and feces and urine excretion were monitored. There was 6 day adaptation period followed by a 4 day collection period.

## **Results:**

The finisher diets used in the trial emulated those fed at the Arkell Swine research station to avoid dietary adaptation. The only difference in the diets was the addition of supplemental phytase to the conventional Yorkshire diet. At the Arkell Swine research barn prior to the trial, the Cassie pigs grew somewhat faster than the Yorkshire pigs, which somewhat skewed the results. During the trial the larger Cassie pigs ate more feed, but there was no difference in weight gains of the pigs because the Yorkshire pigs exhibited compensatory weight gains during the trial. However, the Cassie pigs retained 34% more of the cereal grain phosphorus consumed than did the Yorkshire pigs. Phytase activity in the saliva from Cassie pigs averaged  $160.4 \pm 44.8$  U/ml of saliva although it varied widely over a series of assays for individual pigs, and as well as among pigs. Analysis of ions in urine samples revealed significantly higher contents of phosphorus, sodium, potassium, and chloride and a low content of calcium in Cassie urine, and lower contents of phosphorus, sodium and potassium and a higher content of calcium in the Yorkshire urine. These data in conjunction with a significantly lower blood serum alkaline phosphatase in Cassie pigs as compared to Yorkshire serum document that the Cassie barrows derived an excess of phosphorus from the diet in comparison to the Yorkshire barrows that received a limiting concentration of available phosphorus from the diet, despite the inclusion of phytase.

## **Benefits to the swine industry:**

This data shows that the Cassie line of Yorkshire Enviropig™ is more efficient at digesting cereal grain phosphorus than conventional Yorkshire pigs receiving a diet containing the typical level of phytase supplementation. Therefore, once the Enviropig™ has received regulatory approval, a line of phytase producing pigs is available for commercialization.

## **Acknowledgements:**

Appreciation is expressed to Ontario Pork, the Natural Sciences and Engineering Research Council of Canada and OMAFRA for financial and in-kind support.

# THE KIDNEY PROTEOME OF THE CASSIE LINE OF ENVIROPIG™ & CONVENTIONAL YORKSHIRE PIGS

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## Background.

To assess food safety of the Cassie line of Enviropig™ we have studied the health of the pigs using clinical and performance parameters and the gross chemical analysis of tissues in relation to the same parameters of conventional Yorkshire pigs. To further analyze food safety we conducted iTRAQ analysis of the major food tissues including liver (Golovan et al., 2008), heart, muscle, parotid, serum and skin. In this paper we present the features of the Enviropig™ kidney proteome and comparator Yorkshire proteome. Gender specific and transgenic related differences in the proteomes are also presented.

## Methodology.

Six intact male and six female finisher pigs of each line (conventional Yorkshire or transgenic Cassie pigs), were used for collection of kidney tissue samples. Tagged swine kidney peptides were separated by ion exchange and reverse phase column chromatography and identified by mass spectrum analysis and proteins were assembled using the ProteinPilot software. The ratios of individual kidney proteins in males and females, and transgenic vs. non-transgenic pigs were determined. In addition, the main physicochemical features of the proteins including (Mw, pI, Gravy index), cellular localization, and functional roles were assessed using the programs Gene Ontology and DAVID.

## Results.

We developed a highly confidence (>95%) list of 1606 porcine kidney proteins, which is one of the largest available databases for mammalian species, and is the most comprehensive compendium of porcine kidney proteins. Almost 50 % of the proteins exhibited similarity to human kidney proteins. Functional analysis of the proteins showed that they were involved in a broad range of cellular activities, the major activities including metabolic process, biological regulation, transport, organic acid metabolism, cellular communication, and 18 other functions with fewer proteins in each. A comparison of the ratio of each of the respective proteins of males to females did not show differential expression after correction for the false discovery rate. Similarly, a comparison of the ratio of respective proteins in transgenic kidney and conventional pig kidney did not show differential expression after correction for the false discovery rate. This data supports the thesis that no unintended changes occurred in the development of the Cassie line of Enviropig™.

## Benefits to the swine industry.

iTRAQ analysis of proteins in the kidneys of transgenic Enviropigs and conventional Yorkshire pigs documented that there are no differences in the proportions of the major proteins in kidney tissue. This is consistent with similar studies on liver, muscle and skin.

## Acknowledgments

We are grateful to Derek Smith (Proteomics Centre, University of Victoria, BC) for help with iTRAQ analysis, and Dr. Flavio Schenkel (Animal & Poultry Department, UofG) for his kind support with the data analysis. This research was supported by funding from Ontario Pork, AFMNet, OSTAR, and OMAFRA.

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## ENVIRONMENTAL NITROGEN LOSSES FROM PORK PRODUCTION

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### BACKGROUND

Swine manure is an important N source, but mismanagement of swine manure can increase the risk of water and air pollution. Although the general mechanisms of manure N transformations and losses are known, the actual proportions of different loss mechanisms with reference to application time (fall vs. spring); manure type (solid vs. liquid) or soil hydrologic soil groups remain to be elucidated. We used liquid swine manure labeled with  $^{15}\text{N}$  in both inorganic and organic fractions to study manure N transformations and to directly measure manure N recovery by corn, retained in the soil, and losses by leaching and gaseous emissions.

### METHODOLOGY

Field experiments were conducted on two soil types (a well drained fine sandy loam and an imperfectly drained silt loam). Manure enriched with  $^{15}\text{N}$  was produced by feeding swine with diets containing  $^{15}\text{N}$  enriched corn and soybean. Experimental treatments included three manure application timings: late-fall, spring pre-plant, side-dress and a control. Corn was planted in May of 2006 and 2007. Repeated soil sampling and drainage water sampling were carried out from November 2005 to October 2007. Corn grain and stover were sampled at physiological maturity for total N and  $^{15}\text{N}$  analysis. The total N, mineral N ( $\text{NH}_4$  and  $\text{NO}_3$ ) and  $^{15}\text{N}$  enrichment in soil samples, and mineral N and the  $^{15}\text{N}$  enrichment in the drainage water were determined.

### RESULTS

Manure N uptake by corn (*Zea mays* L.) was significantly lower with fall application than with two spring applications (14-18% vs. 30-38% of applied) in both soil types. Manure application increased total N leaching (30-43 vs. 27 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the control), especially with fall application. Manure contributed 18-25% of the total N leached in the fine sandy loam and 8-10% of the total N leached in the silt loam. Application timing did not affect manure N leaching in the silt loam, which ranged between 3 to 5% of applied. In the fine sandy loam, fall application resulted in significantly higher manure N leaching (15% of applied) than with two spring applications (8-10% of applied). Unaccounted losses, assumed to be in gaseous forms, over 6 months following fall application were higher in the silt loam than in the fine sandy loam (39 vs. 21 kg N ha<sup>-1</sup>). Estimated  $\text{NH}_3$  volatilization losses were low (3-4 kg N ha<sup>-1</sup>), hence denitrification was probably the main mechanism for gaseous N losses. Denitrification to leaching ratio for fall manure N was 7:1 in the silt loam and 2:1 in the fine sandy loam. To maximize manure N use by corn and minimize environmental N losses, spring or side-dress application of liquid swine manure is recommended, particularly in well drained soils.

### BENEFITS TO THE SWINE INDUSTRY

Our results can be used to increase farmers' awareness of environmental losses, as the  $^{15}\text{N}$  tracers used are directly linked to manure application.

### ACKNOWLEDGEMENT

Funding was provided by the Ontario Ministry of Agriculture, Food and Rural Affairs and Ontario Pork. We thank two farmer co-operators for allowing us to conduct the experiment on their farms.

## **Monitoring of *Salmonella*, *Campylobacter*, *Yersinia enterocolitica*, *E. coli* O157, and *Listeria monocytogenes* on a subset of Canadian swine farms**

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### **Background**

In order to reduce the risk of foodborne diseases it is important to monitor the prevalence of potential human pathogens at all stages of the food chain.

### **Objectives and Methods**

The objective of the present study was to investigate the occurrence of major bacterial foodborne pathogens on pig farms. In total, 359 samples from manure storage tanks (91) and pooled fecal samples (268) obtained from finisher pigs (110), sows (78), and weanlings (80) were collected and cultured for bacteria associated with foodborne disease.

### **Results and Discussion**

*Campylobacter sp.*, *Salmonella sp.*, *Yersinia enterocolitica*, *Escherichia coli* O157 and *Listeria monocytogenes* were isolated from 36.5%, 31.5%, 5.8%, 3.3%, and 3.3% of samples, respectively. All *E. coli* O157 isolates found on 10 farms were tested but none were determined to be *E. coli* O157:H7. The 113 *Salmonella* isolates were recovered on 24 farms and the four most common serovars were *S. Typhimurium* var. Copenhagen (31.0%), *S. Derby* (12.4%), *S. Typhimurium* (10.6%), and *S. Agona* (10.6%). Of 131 *Campylobacter* isolates recovered on 21 farms, 118 isolates were *C. coli* and 13 isolates could not be speciated. The sero/biogroups of *Y. enterocolitica* were O3/biotype 4 (16 isolates), O6,30/biotype 1A (three isolates), O5/biotype 1A (one isolate), and O8/biotype 1B (one isolate). Prevalence varied with season and age group.

### **Significance to the industry**

These findings provide baseline information on the distribution of important zoonotic pathogens in swine and indicate that pigs should be considered as a possible source of foodborne diseases in humans.

### **Funding**

OMAFRA, Ontario Pork, University of Guelph-OMAFRA Sustainable Production Systems, PHAC, Agriculture Canada.

## Vaccination to control *Salmonella* shedding and improve growth in pigs

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### Background

*Salmonella* Typhimurium is an important foodborne pathogen and can cause clinical disease in pigs. This organism can be found on many pig farms in Ontario. The on-farm control of *Salmonella* Typhimurium is difficult.

### Objectives and Methods

The objectives of the present study were i) to determine if either an autogenous *S. Typhimurium* vaccine or a commercial live *S. Choleraesuis* vaccine could reduce the prevalence of *Salmonella* shedding in market weight hogs, ii) to determine if *Salmonella* shedding has an impact on the weight gain of pigs. The trial was conducted on one farrow-to-finish pig operation with a history of clinical and sub-clinical salmonellosis. Nine batches of weaned pigs, with 350 pigs in each batch, were randomly assigned to 1 of 3 treatment groups: injection with an autogenous *S. Typhimurium* vaccine, in-water administration of a commercial *S. Choleraesuis* vaccine, or an untreated control group. Pooled fecal samples were collected by-weekly from finisher pens and cultured for *Salmonella*. In each batch, 30 pigs were weighed in the nursery stage and again when marketed.

### Results and Discussion

In the multilevel analysis, the *S. Choleraesuis* vaccinated pigs were more likely to shed *Salmonella* compared to pigs vaccinated with *S. Typhimurium* vaccine and the chance of *Salmonella* shedding in both vaccinated groups was higher than in the control group. However, the largest decrease in the prevalence of *Salmonella* shedding over the entire production stage, i.e. from nursery until marketing, was seen in the group vaccinated with the *S. Typhimurium* autogenous bacterin. The multilevel analysis showed that the pigs from pens with the highest *Salmonella* shedding had a low average daily gain.

### Benefit to Swine Industry

The most significant finding in this trial is that growth rate might be improved if *Salmonella* shedding is prevented. The *S. Choleraesuis* vaccine delivered in the water was easier to use than the injectable *S. Typhimurium* vaccine but didn't appear to work as well against *S. Typhimurium* infection. The development of an oral *S. Typhimurium* vaccine for swine may hold promise.

### Funding Support

OMAFRA Food Safety program, Gallant Custom Labs and Intervet Schering-Plough Animal Health

## Antimicrobial resistance in *Salmonella* on Ontario swine farms

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### Background

Antimicrobial resistance is a concern for pork producers because it is a public health issue but as well resistance to antibiotics may lead to problems in treating pigs with economically important bacterial diseases.

### Objectives and Methods

The objective of this study was to describe the farm-level prevalence of antimicrobial resistance in *Salmonella* isolated on Ontario swine farms between 2001 and 2006.

### Results and Discussion

No resistance was determined to amikacin and ciprofloxacin and only one nalidixic acid resistant isolate was recovered in 2001. Only 1% of the isolates were resistant to ceftiofur, ceftriaxone, apramycin, cephalothin, amoxicillin/clavulanic acid, ceftiofur, and gentamicin. Resistance to ceftiofur, ceftriaxone, and apramycin was observed only in the last year of the study. The most frequent resistance was seen against sulfisoxazole (45.1% of isolates), tetracycline (43.4%), streptomycin (42.5%), spectinomycin (41.6%), chloramphenicol (36.3%), and ampicillin (35.4%) followed by neomycin (23%), kanamycin (23%), and nitrofurantoin (18.6%). Resistance to the cephalosporins and carbadox was found on 2 farms each in the last year of the study. Three groups of farms were defined based on *Salmonella* status for each year of the study. A farm was classified either as Group 1 if no *Salmonella* were recovered during the entire study period, as Group 2 if *Salmonella* without antimicrobial resistance were isolated, or classified as Group 3 if *Salmonella* with antimicrobial resistance were cultured. When defining these 3 groups for the entire study period, 44 (39%), 30 (27%), and 39 (35%) farms were categorized into Group 1, Group 2, and Group 3, respectively.

### Significance to the industry

Significant trends were detected in farm-level prevalence of antimicrobial resistance over the 5 years of this study. These findings indicate that monitoring over time may be useful to detect changes in antimicrobial resistance patterns on swine farms.

### Funding

OMAFRA, Ontario Pork, Agriculture Canada, and PHAC.

## **A longitudinal study of MRSA colonization in pigs**

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### **Background**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is major concern to the medical community. It is estimated that there were over 94,000 cases of invasive MRSA infections in the United States in 2005, with over 18,000 of these cases ending in death. Pigs like other domestic animals can be a reservoir for MRSA.

### **Objectives and Methods**

The goal of this project was to determine when in a pig's life they become colonized with MRSA and secondly to attempt to identify likely risk factors contributing to colonization.

One farm (not using antibiotics) previously identified as having MRSA in pigs was enrolled in this longitudinal study. Nasal swabs were collected from sows 2 weeks prior to entering the farrowing rooms and the presence of MRSA. Five sows from the 46 sampled were determined to be MRSA positive, of which 3 of these sows were included in the trial with seven additional MRSA negative sows. Nasal swabs were collected from individually identified piglets from the 10 sows at 1, 3, 7, 14, 21, 28, 42, 56 and 70 days of life to determine the presence or absence of MRSA.

### **Results and discussion**

One hundred piglets started the study. Ninety survived until the end of the sampling period. The prevalence of MRSA colonization on days 1, 3, 7, 14 and 21 was 1%, 6.2%, 8.5%, 4.4%, 20%, respectively. Overall, MRSA was isolated from 34.5% of piglets at one or more times prior to weaning. MRSA was identified from 34%, 64%, 50% and 41% of pigs on days 28, 42, 56 and 70, respectively. MRSA was isolated from 86% of pigs during the postweaning period, and 79% overall. Of piglets surviving to weaning 84% of piglets from negative sows and 100% of piglet from positive sows were identified as colonized with MRSA at one point. A piglet from a MRSA positive sow was 1.4 times more likely to be colonized with MRSA than a piglet with a MRSA negative dam.

### **Significance to the industry**

Although piglets born from MRSA colonized sows were more likely to quickly pick up the MRSA, piglets in other litters also became positive, particularly at weaning when piglets were mixed. It is unlikely that MRSA can be controlled by screening and segregation of positive animals.

### **Acknowledgments**

The authors would like to thank Joyce Rousseau and the Weese Lab for technical support and the participating pork producer for assistance with sample collection and the use of his farm. Funding was obtained from Canadian Pork Council and NSERC.

## FUNCTIONAL GENOMICS OF DISEASE RESISTANCE IN PIGS

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### BACKGROUND

We have been looking for genetic variations associated with the occurrence or severity of pneumonia in young pigs infected with common agents involved in the respiratory disease complex. Various single nucleotide polymorphisms (SNPs) were identified in genes for some innate immune lectins (mannan-binding lectins (MBL), ficolins and surfactant protein A (SP-A)) that bind to saccharide patterns on the surfaces of some bacteria and viruses<sup>1</sup>. Expression of porcine MBL-C was linked to polymorphisms in the MBL2 gene promoter regions<sup>2</sup>. One allele (MBL2 -1081A) that was most associated with low MBL-C expression was significantly more frequent in pigs with various pneumonias at post-mortem<sup>2</sup>. Pigs have variable amounts of ficolin  $\alpha$  that binds and neutralizes the PRRS virus at physiological concentrations<sup>3,4</sup>.

### METHODOLOGY

A panel of 27 SNPs in 13 porcine innate immune genes, including MBLs, ficolins, and SP-A was developed on the basis of expected roles in resistance to disease<sup>4</sup>. This panel was used to genotype pigs diagnosed with various common diseases (n = 464), in comparison with healthy pigs of various breeds (1283), and pigs (440) in a single farm where growth rates were measured. Associations between mutant genotypes and susceptibility to and severity of disease (graded histologically in PRRSV positive pneumonia cases) were identified.

### RESULTS

Several alleles including the MBL-C -1081A allele were associated with pneumonias at postmortem but were not significantly associated with severity of pneumonia or growth rate. Two SNPs linked on chromosome 14 (MBL-A 271T, SP-A 439A) were associated with more severe necrosis and inflammation, however due to the small number of pigs available for analysis to date (75), these results were not significant. These genotypes were not related to the occurrence of pneumonia, or growth performance in weaned pigs. A galectin-4 allele (GAL4 96T) was significantly more frequent in pigs with pneumonia, enteritis septicemia, and some bacteria (*Streptococcus suis*, K88+ *E. coli*, *Mycoplasma* spp.) and viruses (porcine circovirus-2).

### BENEFITS TO THE SWINE INDUSTRY

These studies have identified polymorphisms in innate defense proteins that might affect the occurrence or severity of pneumonia or other infectious diseases. These SNPs could be useful in marker assisted selection or included in genetic selection panels. Ultimately the benefit of this ongoing project would be to create genetic pig lines with higher innate disease resistance which could decrease production costs associated with impaired growth performance due to subclinical infections and therapeutic antibiotic use, while improving animal welfare. Further studies on large samples of pigs with available production data, as well as determining if other disease-associated SNPs exist in other innate immune genes are required to identify the full array of gene defects involved in impaired disease resistance and to validate their impact on disease resistance and production.

### ACKNOWLEDGEMENT

This work is a collaboration with Drs. Natalie Keirstead and Robert Friendship, with support from Ontario Pork, OMAFRA, NSERC and CIHR.

References: 1. Lillie BN, Brooks AS, Keirstead ND, Hayes MA. Vet Immunol Immunopathol. 2005. 108: 97-100. 2. Lillie BN, Keirstead ND, Squires EJ, Hayes MA. Dev Comp Immunol. 2006. 31: 830-846. 3. Keirstead ND, Lee C, Yoo D, Brooks AS, Hayes MA. Antiviral Res. 2008. 77: 28-38. 4. Keirstead ND. PhD Thesis, University of Guelph. 2007.

## Evaluation of the Ontario Swine Veterinary-based Surveillance System (OSVS)

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### **Background:**

Recent disease outbreaks in the Ontario swine industry involving Porcine Circovirus type II (PCV-2), swine influenza (H3N2) and Porcine Reproductive and Respiratory Syndrome virus (PRRS) illustrate the need for an early disease detection system. Traditionally, we have relied on passive surveillance from regional diagnostic labs, but this may not be the best approach for the early detection of outbreaks. In contrast, practicing veterinarians are often the first to detect the initial cases of novel infectious diseases. Consequently, we undertook a twelve-month pilot study to determine the feasibility of a veterinary-based surveillance system for Ontario. This pilot program was evaluated based on the level of compliance among practitioners, and the timeliness and quality of data submitted to the program. Currently, practitioners continue to participate in the program and program evaluation is on-going.

### **Methodology:**

Seven veterinarians from five clinics recorded and transmitted data from July 1, 2007 to June 31, 2008 using either paper forms or a personal digital assistant (PDA). The practitioners summarized the number of farm visits and calls according to the body systems affected and the manner in which production was affected.

### **Results:**

For the pilot study, the average time to availability of data was 21.7 days and the quality of compliance and timeliness was variable among veterinarians and months. The completeness of fields in the form ranged from 82-99%. Actual estimates of morbidity and mortality for disease visits/calls were rarely recorded. Over the course of the pilot study, almost 25% of swine farms in Ontario were visited. After the pilot study, an increase in coverage of 7% was observed from June 2008 to March 2009 and timeliness improved to 18 days. However, timeliness and compliance continue to be highly variable among veterinarians.

### **Benefits to the swine industry:**

It is feasible to administer a swine veterinary-based surveillance system in Ontario that is acceptable to practitioners, and can produce useful surveillance data. The use of friendly recording systems adapted to each clinic's management system helped improve timeliness and compliance. However, financial incentives may be needed to achieve a "real-time" surveillance system using practitioner data.

### **Acknowledgments**

Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA), Food Safety Initiative of the Canadian Food Safety & Quality Program of the Agriculture Policy Framework and participating veterinarians

## **Clinical signs due to PRRS in Ontario Swine Herds**

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### **BACKGROUND**

Clinical signs of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) include severe acute reproductive losses and chronic high morbidity and mortality in nursery to finisher pigs in endemically affected herds. In 2004 and 2005, a new wave of clinical problems due to PRRSV hit the Ontario industry.

### **METHODOLOGY**

In total, 455 sites where producers had received positive PCR tests for PRRSV at the Animal Health Laboratory were included in the study. Information about the clinical signs of disease during the PRRS problem was gathered using a telephone survey. Producers were asked to describe the abnormal signs of concern.

### **RESULTS**

The clinical problems and the proportion of herds with the problem included mortality of nursery (69%), nursing (52%), finisher (46%) pigs and sows (28%). Approximately half of the herds experienced sows off-feed, abortion, stillbirths, and weakborn pigs. Nursery and finisher respiratory disease occurred in 65% and 47% of the herds. Farrow-to-wean sites were more likely to have abortions than farrow-to-feeder or farrow-to-finisher units. Finishing only sites were more likely to have mortality and respiratory disease in the finisher pigs than farrow-to-finish sites.

### **BENEFITS TO THE SWINE INDUSTRY**

There was a new outbreak of clinical PRRSV in the Ontario swine population from late 2004 onward. Describing the clinical problems due to the outbreak helps to understand the extent of the problem. This information will be used in future research to understand the link between clinical signs and genotype of the virus identified in each herd. The fact that farrow-to-wean units experienced more abortions and offsite finishers experienced more finisher pig problems, helps us to understand how the progress of the disease differs by farm type. It is likely that the virus is maintained in the farrow to finish units, resulting in a longer standing immunity whereas in off site finisher barns, the pigs experience an acute outbreak that affects a large number of pigs at once.

**Funding** – Ontario Pork and the University of Guelph – OMAFRA Sustainable Production Systems Program

## Association between clinical signs of PRRS and PRRS control strategies

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### BACKGROUND

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes significant economic losses to producers. Clinical signs include severe acute reproductive losses and chronic high morbidity and mortality in nursery to finisher pigs in endemically affected herds. Producers and veterinarians need to determine the best disease control protocols in the face of an outbreak.

### METHODOLOGY

Producers in Ontario who had submitted samples that tested positive for PRRS using the PCR test at the Animal Health Laboratory, University of Guelph between September 2004 and August 2007 were eligible to participate. In total, 455 sites were included. Information about the clinical signs of disease during the PRRS problem and control strategies employed was gathered during a telephone survey.

### RESULTS

More than half of the herds with sows chose to use vaccines using a variety of protocols. Use of commercial PRRS vaccine in sows and/or gilts was associated with a decreased risk of weakborn pigs (OR=0.56), pre-weaning mortality (OR=0.54) and use in gilts reduced nursery barn mortality (OR=0.45) ( $P<0.05$ ). Use of serum inoculation in gilts and sows increased sow mortality (OR=2.9) and sows off feed (OR=2.6).

PRRS Control Strategy	% of sites using the strategy
Vaccinate gilts at arrival or before breeding	44
Vaccinate pregnant sows	18
Vaccinate sows at weaning	26
Any use of vaccine in a herd with sows	53
Vaccinate nursing pigs or nursery pigs	2
Serum inoculation in gilts and/or sows	11
Serum inoculation in nursery pigs	0.4
Feedback to gilts or sows	23
No control program used	27

### BENEFITS TO THE SWINE INDUSTRY

Producers and veterinarians are faced with the choice of vaccination, serum inoculation, feedback or letting the disease run its course when a herd has clinical problems due to PRRSV. Vaccination was still the preferred course of action over serum inoculation or feedback, although 27% of herds chose to use no control program. Serum inoculation may result in an increase in sow deaths and sows off feed.

**Funding** Ontario Pork and the University of Guelph – OMAFRA Sustainable Production Systems Program

## Genotypes of PRRS in Ontario Swine Herds from 2004 to 2007

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### BACKGROUND

In 2004 and 2005, a new wave of clinical problems due to PRRSV hit the Ontario industry. It was unclear whether the outbreak was due to new genotypes infecting the herds or whether there were a high number of naïve farms newly infected with genotypes that had been in the population for a long time.

### METHODOLOGY

Producers who submitted samples to the Animal Health Laboratory between 2004 and 2007 were eligible to participate in the study. In total, 455 sites where producers had received positive PCR tests for PRRSV at the Animal Health Laboratory were included. The genetic sequence of the ORF 5 region of virus was determined and then the RFLP type was determined either using enzymes or was predicted using the computer.

### RESULTS

The most common RFLP identified, representing 25%, 15% and 12% of the data respectively, were 1\_4, 252 and 134. The cut pattern 252 and 142 represent the modified live vaccine viruses. During the study, 184 emerged in Ontario as a new RFLP type. It represented 4% of the barns included in this study. Although 1\_4 had been identified in Ontario before the start of the study, the prevalence and the relative prevalence of this type increased significantly.

RFLP	Number of Sequences	Proportion of all sequences (%)
1_4	111	25
252 <sup>a</sup>	65	15
134	53	12
1_2	34	8
132	24	5
142 <sup>b</sup>	22	5
182	21	5
184	16	4

### BENEFITS TO THE SWINE INDUSTRY

The new outbreak of clinical PRRSV in the pigs that began in 2004 was, at least in part, due to the presence of two new types of PRRSV that infected the pigs in Ontario. These types had been fairly prominent in Quebec, with each type representing over 20% of the isolates reported. It is possible that these types were imported with pigs coming from that province.

### FUNDING

Ontario Pork and the University of Guelph – OMAFRA Sustainable Production Systems Program

# Activity of Microencapsulated Carvacrol against *Escherichia coli* with K88 Pili

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## BACKGROUND

Essential oils (EOs) are volatile components of plants that have been extensively studied and used in a wide range of food systems to increase the safety and shelf life of foods, but little has been done in the feed applications. EOs offer an alternative to antibiotics in feed due to the characteristic odor or flavor of the plant from which they are extracted and strong antimicrobial activity some EOs have. However, the early absorption of EOs in animal upper guts and their tendency to bind to feed has been shown to diminish *in vivo* activity. An effective delivery of EOs to the intestines is thus required to target bacterial pathogens and promote growth.

## METHODOLOGY

Alginate solution (2% w/v) containing 20% (w/v) of carvacrol and 0.05% (w/v) tween-80 was blended for emulsification, then extruded into a collecting water bath containing 2% (w/v) CaCl<sub>2</sub> using an Inotech Encapsulator IER-50 with a 500 µm nozzle (Inotech Inc. Maryland, USA). The resulting microcapsules were allowed to harden in the CaCl<sub>2</sub> solution for 30 min then drained, rinsed with water and dried at room temperature.

Prepared microcapsules (wet and dried) were tested for stability, release profiles, and antibacterial activity in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Antimicrobial assays were performed by measuring the growth (OD<sub>600</sub>) of *E. coli* K88 in the presence and absence of encapsulated carvacrol.

## RESULTS

Carvacrol was used as a model essential oil and successfully encapsulated in microcapsules made from Ca-alginate hydrogel using an emulsion-extrusion technology with high encapsulation efficiency. This encapsulation method did not compromise the antimicrobial activity when tested against *E. coli* K88 in a culture medium, as well as in a simulated gastrointestinal model. In the simulated gastrointestinal model, less than 20% of encapsulated carvacrol was released in the simulated gastric fluid; the rest was nearly completely released in the intestinal fluid after 6 h incubation. Encapsulated carvacrol needs to be further tested on animals for its activity in the gut. The encapsulation technology can be extended to other EOs with similar properties.

## BENEFITS TO THE SWINE INDUSTRY

Development of EOs into a viable alternative to antibiotics in feed would contribute to mitigating the use of dietary antibiotics and reduce the burden of bacterial pathogens in swine production.

## ACKNOWLEDGEMENT

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# **Porcine IgE in the context of experimental food allergy: Purification and isotype-specific antibodies**

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## **Background:**

Measurement of allergen-specific immunoglobulin E (IgE) is a common practice in investigation of allergy. It has not been possible to measure porcine IgE due to unavailability of anti-porcine IgE.

## **Objective:**

This study was undertaken to purify and characterize porcine IgE from sera of allergic pigs, identify heterologous anti-IgE reactive with pig IgE and to use purified heavy (H) chain of porcine IgE to generate rabbit anti-IgE.

## **Materials and Methods:**

A four step process for purification of porcine IgE is reported using ammonium sulphate precipitation, Protein-G affinity chromatography, DEAE cellulose anion-exchange chromatography and polyacrylamide gel electrophoresis (SDS-PAGE) to obtain IgE H chain. The resultant IgE was evaluated for purity using SDS-PAGE and immunoreactivity was detected by Prausnitz-Küstner (PK) tests and passive cutaneous anaphylaxis (PCA) with the allergen, crude peanut extract, used to induce experimental allergy. Cross-reactivity with anti-mouse and anti-human IgE antibodies were confirmed in western blot and enzyme-linked immunoassays (ELISA). The H chain of IgE was excised from SDS-PAGE gels and used to develop rabbit anti-porcine IgE antisera.

## **Results:**

Antiserum obtained from rabbits immunized with porcine IgE, as well as heterologous murine and human-specific anti-IgE, induced reverse cutaneous anaphylaxis (RCA) in pig skin and detected allergen-specific IgE in ELISA but did not react with IgG H chain in western blots. These results confirm allergy-associated bioactivity of porcine IgE and describe both homologous and heterologous anti-pig IgE suitable for use in allergen-specific and other assays.

## **Conclusions and Benefits to the Swine Industry:**

This will enhance utility of pig allergy models and provide an additional measure of type-2 immune response in pigs.

## **Acknowledgements:**

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# Studies of the Interactions among ETEC K88, Porcine Gut Cells, and Lactic Acid Bacteria

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## BACKGROUND

Enterotoxigenic *Escherichia coli* (ETEC) cells with the K88 fimbrial (K88<sup>+</sup> ETEC) adhere to the pig gut and cause diarrhea in newly-weaned piglets. We have previously selected some LAB isolates from the pig gut that were inhibitive to *E. coli* K88, tolerant to O<sub>2</sub>, low pH, and high bile salt, and able to bind to porcine gut epithelial cells (IPEC-J2). The gut cells have recently been established as an *in vitro* model for studies of microbe-host interactions. The objective of the present study was to study the interactions among the pathogen, LAB isolates, and gut cells and determine the molecular mechanisms underlying the interactions.

## METHODOLOGY

IPEC-J2 was pig neonatal jejunal epithelial cells and grown in DMEM-F12 (1:1) at 37°C. Epithelial cell death was estimated by trypan blue staining. Cytotoxicity was determined by measuring released lactate dehydrogenase (LDH). The expression of toxin genes of *E. coli* K88 JG280 was quantified by Q-PCR. AI-2 assay was performed by measuring bioluminescence.

## RESULTS

K88 JG280 at 10<sup>8</sup> CFU/ml or a low concentration caused more IPEC-J2 cell damage than at 10<sup>9</sup> CFU/ml. Microscopic observation, trypan blue staining, and cytotoxicity assay all revealed that K88 JG280 at 10<sup>8</sup> CFU/ml or a low concentration caused more IPEC-J2 cell damage than at 10<sup>9</sup> CFU/ml, regardless that IPEC-J2 cells were cultured in DMEM-F12 or D-MEM medium (without sodium pyruvate). The *STb* toxin was suggested to be involved in the cell damage, although further verification is required.

K88 JG 280 at different concentrations differentially expressed AI-2 activity. The AI-2 activity was differentially expressed in the cultures of K88 JG280 at 10<sup>8</sup> and 10<sup>9</sup> CFU/ml during 3 h incubation. Given a much higher AI-2 activity produced in the bacterial culture with 10<sup>9</sup> than 10<sup>8</sup> CFU/ml and the observation over the gene expression of the *STb* toxin, AI-2 may have negatively regulated the toxin gene expression. Further studies are required to verify the hypothesis.

LAB isolates were able to reduce the cytotoxicity of K88 JG 280. A few LAB isolates have been shown to significantly reduce the cytotoxicity of K99 JG280 (10<sup>8</sup> CFU/ml) to IPEC-J2 cells when incubation with the pathogen and gut cells. The protection effect of LAB isolates in relation to the toxin and AI-2 production will be investigated.

## BENEFITS TO THE SWINE INDUSTRY

Elucidating the molecular mechanisms of interactions among *E. coli* K88, porcine gut cells, and LAB isolates would contribute significantly to developing control strategies of the pathogen and to further improving swine production and pork safety.

## ACKNOWLEDGEMENTS

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# Is encapsulation the solution to the development of alternative treatment?

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## BACKGROUND

Pathogenic bacteria in pig gastrointestinal tract (GI) such as *Salmonella* and *E. coli* cause diseases in both animals and humans. Control of enteric bacterial pathogens on pig farms would be beneficial with respect to public health and production efficiency. Extensive use of antibiotics to control pathogens has led to growing consumer concerns, thus there is an urgent need for viable alternatives to antibiotics. Progress has been made in the use of natural products including essential oils (EOs), probiotics and bacteriophage for controlling enteric bacterial pathogens. However, the lack of consistent efficacy and stability of these products in animal trials limited their successful application. One of the factors identified that could be responsible for the failure of these products is the loss of antimicrobial activities during the passage through upper GI tract; as a result, insufficient active agents can reach the small intestine to exert their antibacterial activities. Studies have shown that EOs may be mostly absorbed in the stomach; stomach acids, bile and digestive enzymes may cause damage to probiotic bacteria and bacteriophages. To overcome these problems, our research is focused on developing microencapsulation technologies for targeted delivery of essential oils and bacteriophage.

## METHODOLOGY

Different encapsulation techniques and polymers have been considered and tested for delivering essential oils and bacteriophages because essential oils from various sources are different in chemical structures and physical properties; bacteriophages are usually sensitive to heat, pressure and organic solvents. The technologies employed in the current research include emulsion-extrusion and lipid melt-solidifying; the polymers used include natural polysaccharides, proteins, fatty acids, and their mixture.

## RESULTS

1. Microencapsulation of two types of phages were developed (Ma et al, 2008, Appl Envir Microbio, 74:4799-), which enabled a large proportion of phages to remain bioactive in a simulated GI environment compared to the free phages. In addition, phages were completely released from the microcapsules upon exposure to simulated intestinal fluid within 6 hours.
2. A model essential oil carvacrol was successfully encapsulated giving satisfactory stability and release profiles in simulated GI conditions. Encapsulation of another essential oil, cinnamon oil is in good progress.
3. Future step is to test developed encapsulated phages and essential oils in pigs separately or in combination of both agents.

## BENEFITS TO THE SWINE INDUSTRY

The success of the research will provide alternatives for controlling *Salmonella* and *E. coli* in swine production, thus increasing production efficiency, meat safety and consumers' confidence in Canadian pork products.

## ACKNOWLEDGEMENT

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# Enhancing intestine development of early-weaned pigs with EGF-expressing *Lactococcus lactis*

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## BACKGROUND

Stress and incomplete gastrointestinal development in early-weaned piglets represent significant challenges in commercial swine farming. As the use of in-feed antibiotics continues to decline it is necessary to develop alternative methods to stimulate intestinal development and provide disease resistance. Orally ingested recombinant epidermal growth factor (EGF) has been shown to remain biologically active in the gastrointestinal tract as well as improve intestine development and resistance to pathogen infection, and reduce the incidence of diarrhea. We have previously shown that the food-grade bacterium *Lactococcus lactis* can express biologically active EGF after ingestion by early-weaned mice.

## METHODOLOGY

Pigs were orally gavaged twice daily with M17 broth containing EGF-expressing *L. lactis*, *L. lactis* alone, recombinant EGF as a positive control, or broth alone as a negative control. At the end of the 14-day treatment period, all pigs were sacrificed and tissue was collected and snap-frozen in liquid nitrogen until further analysis was performed.

## RESULTS

Administering EGF-expressing *L. lactis* as a feed supplement to early-weaned piglets significantly improved intestinal development, which was evident by significantly increased villi height in the duodenum and jejunum, as well as intestinal length. Immunohistochemistry using antibodies against proliferating cell nuclear antigen further revealed that the proliferation of intestine cells was significantly increased by the EGF-expressing *L. lactis*. While the experiment period was too short to observe statistically significant differences in weight gain, pigs treated with EGF-expressing *L. lactis* showed a decrease in the incidence of diarrhea. Probiotic effects of supplementing diets with *L. lactis* alone were also observed, further supporting the use of naturally occurring intestinal microbes as desirable vectors for recombinant protein delivery.

## BENEFITS TO THE SWINE INDUSTRY

These results provide the first demonstration of using biologically safe bacteria as a cost-effective approach for the administration of therapeutic antibiotics to farm animals.

## ACKNOWLEDGEMENTS

This work was supported by Ontario Pork, Natural Sciences and Engineering Research Council (NSERC), Agriculture and Agri-Food Canada. The authors wish to thank Julia Zhu, Doug Wey, James Haley, Dan Columbus for excellent technical assistance in the animal trials. Ping Kang is the recipient of a Visiting Student Scholarship from Chinese Scholarship Council through the MOE-AAFC Ph.D. Program.

## **Screening for neutralizing antibodies against bovine viral diarrhea virus (BVDV) in Ontario swine herds: looking for pestivirus**

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### **Introduction**

The search for pestivirus antibodies and virus isolates has been quite intensive for over 20 years. Interest in the prevalence and clinical significance of pestivirus in swine herds has recently been highlighted with the documentation of neurologic and reproductive disease outbreaks associated with novel pestiviruses in the USA (1) and Australia (2).

It is well documented that pestiviruses will cross-react with Bovine Viral Diarrhea Virus (BVDV) by virus neutralization and therefore this may be a useful screening test for the presence of novel pestiviruses. Pestivirus has yet to be identified in Ontario swine herds and the current prevalence of BVDV in Ontario swine herds is unknown. It was hypothesised by the authors that recent neurologic and reproductive syndromes identified by the swine field service veterinarians at the Ontario Veterinary College, University of Guelph, may be associated with an unknown pestivirus similar to those in the American and/or Australian reports.

The purpose of this study was to determine the prevalence of antibodies against BVDV in Ontario swine herds as an initial step towards determining the prevalence and potential clinical significance of BVDV antibodies in case herds with neurological and reproductive disease problems in a search for pestiviruses.

### **Methods**

Five-hundred sera collected previously (2005) from 50 different Ontario sentinel swine herds (10 per herd), were tested by virus neutralization (VN) for BVDV-type 1 and BVDV-type 2 by the Animal Health Laboratory at the University of Guelph. Post mortem examinations and testing for BVDV-1 and BVDV-2 by VN from, two Ontario herds experiencing neurologic and reproductive problems of undetermined etiology were conducted.

### **Results**

All 500 samples from the sentinel herds tested negative (titres < 1:2) for BVDV-type 1 and BVDV-type 2. Herd records reported that cattle were present on 36% of the farms. On the remaining 64% of the farms only pigs were present. The two case herds tested negative for BVDV-1 and BVDV-2 by virus neutralization and post mortem results including histopathology did not support a diagnosis of pestivirus.

### **Benefit to Swine Industry**

The results indicate that BVDV or cross-reacting pestiviruses do not appear to be present in Ontario swine herds regardless of the presence of cattle on the same premise.

### **Acknowledgements**

Ontario Veterinary College Fellowship, and OMAFRA funding for diagnostic work

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# **A field trial investigating the effectiveness of tulathromycin injection for the control of porcine pleuropneumonia due to *Actinobacillus pleuropneumoniae* on a grower-finisher farm in an outbreak situation.**

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## **Background**

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (APP) results in high mortality and great economic loss in certain herds. The disease can occur acutely so that the producer has little warning that there is a problem. Treatment of pigs in a herd during an outbreak of APP is difficult. Tulathromycin (Draxxin™, Pfizer Animal Health) is a new injectable antibiotic which achieves high concentrations in lung tissue for extended periods of time (7-10 days) and is reputed to be effective in treating APP.

## **Objectives and methods**

The objective of this study was to evaluate the efficiency of tulathromycin in preventing mortality in swine herds due to *Actinobacillus pleuropneumoniae* infection under field conditions. Three trials were carried out on a farm that was subject to an outbreak of APP. The first trial was a comparison of penicillin and tulathromycin, given in an emergency state of outbreak. The second trial was a comparison of penicillin and tulathromycin, in anticipation to an outbreak, and the same groups were given secondary treatment of either ceftiofur or tulathromycin, respectively. In the third trial, groups of pigs were shipped to a finisher barn after the second trial groups, but given one treatment of either ceftiofur or tulathromycin. When penicillin was used it was given at 3 times the recommended dose for 3 days. Both ceftiofur and tulathromycin were given as a single injection and at label dose. All treatments were applied intramuscularly. For all trials, the odds ratio for mortality (both overall reported mortality, and mortality due to APP) of pigs receiving alternative treatment compared to tulathromycin was greater than one.

## **Results and discussion**

In trials 1 and 2, both Cox's regression analysis and survival curves indicated significantly greater expected survival for a pig of average weight treated with tulathromycin over time, as compared to alternative treatment ( $P < 0.05$ ). In trials 2 and 3, tulathromycin was found to significantly ( $P < 0.05$ ) increase average daily gain of pigs. It was concluded that under the conditions of this study and sufficient exposure to APP, tulathromycin would significantly reduce pig mortality and improve growth performance relative to treatment with either penicillin or ceftiofur.

## **Significance to the industry**

This trial demonstrated that tulathromycin appears to be an (APP) (APP) effective treatment for APP and because the drug remains at high concentrations in lung tissue for an extended period of time there is considerable labour savings.

## **Funding**

Pfizer Animal Health

# Environmentally-associated variation in immune response. Implications for pig health

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## Background:

Immune response (IR) of pigs varies by litter and by individual such that ratios of type 1 and type 2 IR differ. Genetics and the environment contribute approximately 20% and 80% to this variation. During pregnancy to ensure the dam does not reject the fetal allograft, the maternal immune system may be biased to a type-2 IR. A type-1 IR is associated with a cell mediated IR (CMIR) and a type-2 IR is associated with an antibody mediated IR (AMIR).

**Hypothesis:** Neonatal pigs are born with a type-2 biased immune response.

## Materials and Methods:

Pigs were IR phenotyped beginning at 14 days of age using an established protocol involving two intramuscular injections of the type-1 antigen *Candida albicans* (CA) and the type-2 antigen hen egg white lysozyme (HEWL) adjuvanted with Quil A. Immune response was measured by double skin fold thickness (DSFT) at 35 days of age by intradermal injection of both antigens at separate sites. Blood was collected at 14, 21 and 35 days of age to measure antibody activity to CA and HEWL. A comparison between previously (A) and recently (B) completed experiments using the above protocol was made.

## Results:

A difference in IR bias was observed between A and B. There was a significant increase in DSFT to CA in both A and B indicating a CMIR, more specifically delayed-type hypersensitivity. However, the response to CA was significantly greater in A. There was a significant increase in DSFT to HEWL in both A and B at one and three hours post injection, indicating an AMIR, or an immediate type hypersensitivity and Arthus reaction respectively. No difference in magnitude of response was observed. A significant increase in frequency of weal and flare to injection of HEWL was observed in experiment B. Antibody activity to CA and HEWL in B was significantly more frequent than in A.

## Conclusion:

Overall, due to increased frequency of weal and flare, increased antibody activity and decreased CMIR to CA, pigs in B were more type-2 biased than pigs in A. It is likely that the environment played a major role in this switch. Environmental factors play an important role in influencing possible type-2 bias of pigs at birth.

## Benefit to Swine Industry:

Understanding environmentally induced variation in IR is important to pig health as variation may influence susceptibility to certain infectious and allergic diseases as well as response to neonatal vaccination. Further studies will examine the ability of heat-killed bacteria and bacterial components to favourably alter IR bias.

## Acknowledgements:

Financial support to B.N Wilkie from NSERC, OMAFRA and Ontario Pork and technical assistance from the Arkel Swine Staff is gratefully acknowledged.

# Oral Exposure of Neonatal Pigs to the Egg Allergen Ovomuroid for Induction of Experimental Food Allergy

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## Background:

Advancement in the understanding of human food-induced allergy is largely dependent on experimental animal models. The most applicable models use outbred species, such as pigs, which respond to food allergens with timing and signs similar to those of humans.

## Hypothesis:

It is hypothesized that repeated oral administration of the egg white protein ovomucoid (Ovm) adjuvanted with cholera toxin, will induce allergy.

## Materials and Methods:

Neonatal piglets were randomly assigned in a split-litter design to two groups, systemic (positive control) or oral (split into low and high dose) sensitization. To test for induction of allergic signs piglets were challenged orally with a mixture of egg white and yoghurt. Outcomes were reported as direct skin tests, clinical signs and serum-related antibody (Ab) isotypes.

## Results:

Variation within and between litters were significant. All systemically sensitized pigs, and some orally sensitized, developed immediate weal and flare reactions after skin test. After oral challenge, systemically sensitized but not orally sensitized pigs displayed clinical signs of allergic hypersensitivity. Serum IgG-related, Ovm-specific Abs were detected in the systemically sensitized pigs and in few orally sensitized pigs. However, IgE-mediated Ab response to Ovm was detected in pigs that showed positive skin test responses, regardless of the sensitization route. Serum Ovm-specific IgE antibody activity was positively correlated to clinical scores, compared to direct skin test, Ovm specific IgG, IgG1 and IgG2 in the positive control group and to direct skin tests in the oral group.

## Conclusions:

Although pigs were orally sensitized to Ovm reflecting a natural route of sensitization, they did not exhibit clinical signs of allergy upon oral challenge. Further optimization of the oral sensitization protocol is required to improve the efficacy of this model.

## Benefit to the Swine Industry:

The ability to sensitize pigs via the oral route will contribute to better understanding of naturally occurring soy allergy in swine.

## Acknowledgements:

Financial support to B.N Wilkie from NSERC, OMAFRA and Ontario Pork and technical assistance from the Arkell Swine Staff is gratefully acknowledged.

# Effects of Dietary Supplementation of Ractopamine and $\alpha$ -Lipoic Acid on Pork Quality

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## Background:

Significant variation in pork quality may be attributed to the rate and(or) extent of post-mortem muscle glycolysis which influences muscle pH decline in the conversion of muscle to meat. Ractopamine (RAC) is a growth promoter that has been found to decrease tenderness. Dietary  $\alpha$ -lipoic acid (LA) supplementation may reduce the rate of post-mortem muscle glycolysis to enhance pork tenderness or reduce the incidence of pale, soft, and exudative (PSE) pork. The purpose of this study was to determine the effects of dietary supplementation of RAC and LA on pork quality.

## Experimental procedure:

Two pig genotypes (purebred Yorkshire, commercial PIC) were included in the trial to represent variability in growth performance and meat quality. Pigs were allocated by breed and gender (barrow, gilts) to pens (2 or 4 pigs/pen; gender balanced) to 1 of 4 management regimens based on the specific finishing diet fed when pigs attained 85 kg body weight. These diets included: 1) Control (CON) finisher, a typical corn/SBM finishing ration, 2) ractopamine (RAC), a higher crude protein finisher containing the  $\beta$ -agonist, ractopamine, 3) Lipoic acid (LA), the standard finisher supplemented with the antioxidant,  $\alpha$ -lipoic, and 4) RAC + LA, a higher crude protein finisher containing RAC and LA. LA was fed at 2 g LA d<sup>-1</sup> for 7 to 14 days pre-slaughter. Pigs were slaughtered at approximately 118 kg BW. Growth performance, carcass characteristics, and meat quality were assessed. Meat quality evaluation of loin chops included assessment of pH, colour (CIE  $L^*a^*b^*$ ), drip loss, shear force and cooking losses.

## Results:

Trait	Evaluation of Meat Quality in Longissimus muscle (loin chops)					
	CON	RAC	LA	RAC+LA	SE	P>F
pH, 1 h post-mortem	6.02	6.10	5.98	6.02	0.467	0.611
pH, 24h post-mortem	5.49	5.53	5.53	5.50	0.249	0.111
Lightness (L*)	50.40	50.4	50.7	49.5	0.660	0.111
Subjective marbling*	1.51	1.70	1.97	1.56	0.098	0.004
Subjective wetness**	1.67	1.88	1.82	1.74	0.747	0.034
Drip loss (%)	9.75	9.73	9.39	9.00	0.418	0.619
Shear force (kg)	4.77	5.43	4.35	5.24	0.242	0.610
Cooking loss (%)	23.50	22.8	22.5	22.3	0.500	0.659

**Take home message:** The benefits of dietary LA on meat quality remain inconclusive. Pre-slaughter LA supplementation did not affect post-mortem muscle pH or colour. However, LA supplementation may enhance marbling, tenderness (lower shear force), and water holding capacity. Feeding LA with RAC (RAC + LA) did not prevent the decrease in tenderness (higher shear force) found when pigs were supplemented with ractopamine (RAC).

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# Cellular signaling pathways affecting pork meat quality; an initial study

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## BACKGROUND

The postmortem processes leading to the development of pH, water holding capacity and meat tenderness in pork meat are critically affected by a number of factors around the time of slaughter, including nutritional status, human interactions and stress. A previous project surveyed the variations in pork quality in a range of commercial operations in Ontario and some of the factors contributing to this. Biochemical studies related stress indicators in the blood at slaughter to variations in quality, and also showed some negative correlations between the activity of cytoskeletal proteases (calpains) and subsequent water holding capacity of the meat. It is well known that stress hormones such as epinephrine affect glycolysis in living muscle and glycolytic potential post mortem. It has been speculated that a signaling pathway involving AMP- activated protein kinase (AMPK) is a key sensor/controller of glycolysis and pH development. However, although some previous work has shown cross-reactions between epinephrine reactions and calpain expression in muscle, there is a lack of information on the key signaling pathways that control this.

## METHODOLOGY

Cultures of skeletal muscle myoblast (C2C12) and fibroblasts (NOR-10) cells are grown in Dulbecco's Modified Eagle's Medium with 10% added fetal calf serum. Prior to confluence, cell cultures are challenged with epinephrine for either 2 or 6 hours. The resultant change in AMPK expression is measured by Western Blot. The activity of calpains is determined by standard zymography and total gelatinase activity is measured by a commercial available kit (Molecular Probes).

## RESULTS

This work is still in its initial stages and only preliminary indications are available at this stage. Preliminary work does suggest that there is a definite time course of transiently increased AMPK expression both in muscle fibroblasts and myoblasts following exposure to epinephrine and that changes in the activity levels of expressed proteases takes longer time to occur. In future work we hope to fully document this and also show changes in signaling and enzyme activity related to nutritional inputs and other stressors.

## BENEFITS TO THE SWINE INDUSTRY

A precise understanding of the complex cell pathways ultimately controlling pH development, tenderization and water loss will provide better opportunities for control of unexplained variability in pork quality and so lead to a more consistently good product.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the support of the OMAFRA-University of Guelph partnership agreement for funding of part of this study.

# Optimization for maximum Deoxynivalenol (DON) biotransformation by bacterial isolate Barpee from soil

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## Background

Deoxynivalenol (DON) is considered to be the most prevalent mycotoxin contaminating corn and wheat crops in North America. This toxin is very stable and, therefore, easily enters the human and animal food chains. Among animal species, pigs show a relatively high sensitivity to DON, and, because of the high percentage of wheat and corn in pig diets, swine are at a greater risk of exposure to this toxin. Efforts are being made by the industry to find acceptable ways for converting mycotoxins to non-toxic products using different strategies. Enzymatic biotransformation of mycotoxins by microorganisms is an efficient approach for the reduction of mycotoxin contamination in feeds and food.

## Methodology

With the goal of providing alternative microbial cultures that can be used as a source of mycotoxin detoxifying activity, one bacterial strain from soil was isolated by using an enrichment culture procedure and tested under aerobic conditions. The DON biotransformation ability of the isolate was tested under different conditions including temperature, pH and media composition. The biotransformation of DON was assayed using LC-UV-MS.

## Results

The isolate named “Barpee” transformed DON into at least two chromatographically separable products identified as 3-keto-4-deoxynivalenol and a DON stereoisomer. The transformation performed differently under a variety of incubation conditions. The greatest extent of DON biotransformation was obtained when corn meal broth (CMB) was used as medium, with a pH between 5-7 and a temperature near 30 °C. The isolate survived and retained its biotransforming activity after six months of storage at -80°C.

## Benefits to the swine industry

Currently on the market there are mycotoxin biotransforming feed additives based on live microbes, especially anaerobic ones. Although they have shown promising results in the field, there are some problems such as limitation due to the anaerobic nature of the microbes, and the detrimental effect on the strain by acidifiers, other components and the high temperatures used in feed processing. Having in mind these aspects and based on preliminary results, our group intends to further explore the potential use of Barpee in the feed industry at different levels such as the use of the pure strain in liquid feeding systems, and its enzymes as feed additives.

## Acknowledgements

This work was supported by Agriculture and Agri-Food Canada.

# Isolation of microorganisms from fish digesta for detoxification of vomitoxin (DON)

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## BACKGROUND:

Mycotoxin contamination (e.g. vomitoxin or deoxinivalenol or DON) of feed ingredients has been a serious threat to livestock industry, particularly swine production. A novel strategy to control the problem of mycotoxicoses in animals is the application of microorganisms capable of transforming mycotoxins into non-toxic metabolites. It has been well established that fish harbor microbial populations in their digestive tracts. It is possible that fish intestinal microbes could play an important role in biotransformation of mycotoxins since the gastrointestinal tract is the main route of entry of xenobiotics into fish.

## METHODOLOGY:

The gut content of a catfish from a local stream was collected from alimentary tracts within 10 h of the fish having been caught. They were diluted with distilled water and then used for DON transformation tests. After a serial dilution and single colony pick up, individual isolates were obtained with DON detoxification activities. The DON degradation ability of the microbial cultures was optimized under different environmental conditions for temperature, pH and media. DON and the detoxified products were analyzed by LC-MS after extract with 50% methanol and compared with commercial standard.

## RESULTS:

Gut content from one catfish was able to transform DON to a much less toxic form, de-epoxy DON (DOM-1);

Microbial community C133 was selected from the fish content and was capable of completely transforming DON to DOM-1 at 15 °C after 96 h under aerobic conditions;

Four bacterial isolates obtained from the C133 showed detoxification activity under anaerobic conditions;

The bacterial isolates were still able to completely transform DON to DOM-1 after 10 generations' subculturing in anaerobic conditions at room temperature.

## BENEFITS TO THE SWINE INDUSTRY:

The bacterial isolates are effective and stable, and can be useful in detoxifying DON in contaminated swine feed for an improved swine production. The capability of the bacterial isolates to transform DON at relatively low temperature as compared to microbes isolated from other sources has significantly increased their potential applications in various swine production systems.

## ACKNOWLEDGEMENTS:

Financial support by Agriculture and Agri-Food Canada and the Chinese Scholarship Council is gratefully acknowledged.

# Impact of immune system stimulation on nutrient digestibility and utilization of sulphur containing amino acids

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## Background

Immune system stimulation (ISS) can cause morphological and physiological changes in gastrointestinal tract. However, the impact of ISS on protein and amino acid (AA) digestibility has not been evaluated. The end-products of sulfur amino acid (SAA) catabolism are mainly excreted in the urine. Therefore, urinary sulfur (S) excretion provides a measure of SAA catabolism. A distinctive feature of SAA is the considerable storage of cysteine in non-protein pools, e.g. glutathione (GSH). GSH has lower nitrogen to S ratio (N/S) than body protein. Hence whole body N/S-balance ratio is an indicator of non-protein SAA storage in growing pigs. Objectives of this study were to determine the impact of ISS on apparent ileal digestibility (AID) of AAs and whole body N/S-balance ratio.

## Methodology

Thirty six barrows were fed restricted (800 g.d<sup>-1</sup>) and assigned to three levels of sulfur amino acid (SAA) intake (Table 1) from SAA limiting diets. Following adaptation, eight pigs at each SAA intake level were injected intramuscularly and every 48 h, for seven days, with either saline or increasing amounts of *Escherichia coli* lipopolysaccharide (LPS). Seven-day N and S balance were then conducted. At the end of balance period, pigs were euthanized and digesta were collected for measuring AID of nutrients.

## Results

AID (%) of amino acids were not affected by ISS (ISS- vs. ISS+; methionine 82.0 vs. 79.0, SE 4.0; cysteine 69.2 vs. 61.0, SE 7.9; lysine 82.0 vs. 77.6, SE 5.1; threonine 71.1 vs. 64.3, SE 6.8; isoleucine 80.5 vs. 76.8, SE 5.2;  $P > 0.50$ ). N and S retention increased with SAA intake ( $P < 0.01$ ). Fecal N and S excretion were not affected by ISS and SAA intake level ( $P > 0.05$ ). ISS reduced N retention but had no effect on S retention (Table 1). Whole-body N to S-balance ratio increased with SAA intake, but was reduced by ISS (Table 1). The latter indicates that ISS increases non-protein SAA pool size in growing pigs.

**Table 1.** Impact of immune system stimulation (ISS) and sulfur amino acid (SAA) intake on nitrogen (N) and sulfur (S) metabolism

SAA intake g/d	1.1		2.1		3.2		SE	P value		
	-	+	-	+	-	+		ISS	SAA	ISS×SAA
<i>N metabolism mg/kg BW/d</i>										
Intake	340	327	664	657	798	715	25	0.10	0.01	0.16
Excretion	132	228	231	252	296	344	14	0.01	0.01	0.05
Balance	207	101	443	410	500	376	19	0.01	0.01	0.04
<i>S metabolism mg/kg BW/d</i>										
Intake	38	37	50	49	57	51	2	0.10	0.01	0.30
Excretion	25	27	30	26	30	28	1	0.12	0.03	0.07
Balance	13	10	20	24	27	24	1	0.30	0.01	0.03
N/S-balance	16	10	20	18	19	16	1	0.01	0.01	0.04

## Benefit to swine industry

These results help us better understand the impact of immune system stimulation on nutrient utilization and animal productivity. Pig performance and economic losses, as well as nutrient losses into the environment, due to immune system stimulation can be reduced by adjusting levels of sulfur amino acids.

## Acknowledgments

This research was supported by Ontario Pork, Evonic, OMAFRA and NSERC.

# Estimating fermentative amino acid losses in the upper gut of pigs

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## Background:

Fermentative catabolism of dietary and endogenous amino acids (FAAC) in the upper gut of pigs can result in a significant loss of amino acids (AA) available for protein synthesis and body maintenance functions. To our knowledge, no estimates of FAAC in the upper gut of pigs are available. The objectives of this research were 1) to explore approaches to estimate AA losses due to FAAC in the upper gut of pigs and 2) to determine the impact of dietary fibre and protein content on FAAC.

## Methodology:

Barrows ( $21.6 \pm 2.0$ kg initial BW) were fitted with a simple T-cannula at the distal ileum, a gastric catheter, and two jugular catheters. Pigs were fed one of three dietary treatments: a control diet (16.9% CP), a high-fibre diet (12% added pectin), or a low-protein diet (13.2% CP).  $^{15}\text{N}$  ammonium chloride (0.8 mmol/kg BW/d) and  $^{13}\text{C}$ -urea (0.2 mmol/kg BW/d) were infused intragastrically and intravenously, respectively, for a period of 4 days. Ileal digesta, blood, and urine were collected on day 0, 3, and 4 of the infusion period.  $^{15}\text{N}$  enrichment was analyzed in ileal digesta ammonia and  $^{15}\text{N}$  and  $^{13}\text{C}$  enrichment were analyzed in blood plasma and urinary urea. Conventional isotope dilutions and mass balance calculations were used to determine whole body urea flux, urea recycling into the upper gut, upper gut ammonia flux, and FAAC. A two-compartment model was also developed to estimate minimum and maximum nitrogen (N) flux through an ileal ammonia pool and a plasma urea pool.

## Results:

Ileal ammonia flow was not affected by the inclusion of pectin in the diet (0.47 vs. 0.25 mmol N/kg BW/d;  $P < 0.05$ ) but was decreased when dietary protein content was reduced (0.13 vs. 0.25 mmol N/kg BW/d) when compared to pigs fed the control diet.

Urinary urea excretion was decreased in pigs fed the low-protein diet compared to the control diet (6.20 vs. 14.4 mmol N/kg/d;  $P < 0.05$ ).

Based on conventional isotope dilution and mass balance calculations:

Inclusion of pectin had no effect on urea flux (25.7 vs. 25.3 mmol N/kg/d;  $P > 0.10$ ) or urea recycling (11.3 vs. 12.1 mmol N/kg/d;  $P > 0.10$ ) but increased ileal ammonia flux (198 vs. 134 mmol N/kg BW/d;  $P < 0.05$ ) and FAAC (165 vs. 102 mmol N/kg BW/d;  $P < 0.05$ ).

Lowering dietary protein resulted in lower urea flux (10.3 vs. 25.3 mmol N/kg BW/d;  $P < 0.01$ ) and recycling (3.23 vs. 12.1 mmol N/kg BW/d;  $P < 0.01$ ).

Use of conventional isotope dilutions and mass balance results in unrealistic values for FAAC and ileal ammonia flux (i.e. higher than total N intake).

Based on the two-compartment model:

Estimated nitrogen flow to FAAC, microbial protein, and ammonia absorption was not affected by inclusion of dietary fibre ( $P > 0.10$ )

Lowering dietary protein decreased minimum (2.74 vs. 11.4 mmol N/kg BW/d;  $P < 0.01$ ) and maximum possible ammonia absorption (9.43 vs. 25.3 mmol N/kg/d;  $P < 0.01$ ) and maximum FAAC (8.40 vs. 16.1 mmol N/kg BW/d;  $P < 0.001$ ).

## Benefits to producers:

Manipulating dietary nutrient content (protein and fibre) can have significant impacts on AA economy in swine. Improvements in AA availability for body maintenance functions and protein deposition in pigs will help reduce feed costs, the number one cost in animal production.

## Acknowledgements:

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## Combined usage of corn distillers solubles and corn steep water for liquid fed growing-finishing pigs

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**Background:** Liquid co-products, such as corn distillers solubles (CDS) and corn steep water (CSW), may serve as alternatives for conventional pig feed ingredients. In previous studies the optimum inclusion levels of CDS and CSW in growing-finishing pig diets have been established at about 15% of diet DM. The aim of this study was to establish the optimum dietary inclusion level of a 50:50 mix of CDS and CSW.

**Experimental procedure:** Purebred Yorkshire pigs (n= 144; initial and final BW 31.9; 4 gilts and 4 barrows per pen; 4 or 5 pens per treatment) were liquid-fed according to a 2-phase feeding program and exposed to one of four dietary treatments: (1) **Control** (standard corn and SBM based diets), (2) **Low** (5% CDS + 5% CSW; DM basis), (3) **Moderate** (10% CDS + 10% CSW), and (4) **High** co-products (15% CDS + 15% CSW). The DM contents of CDS and CSW were 31.5% and 49.0%, respectively. CSW was stored at source for at least two weeks prior to feeding to allow conversion of soluble sugars to lactic acid and incubation with phytase to release phytate phosphorus. Feed delivery (water to feed DM 2.6:1) was computer controlled using feed sensors in the troughs. Pigs had free access to additional water.

### Results:

	<b>Control</b>	<b>Low</b>	<b>Moderate</b>	<b>High</b>			
Treatment	0% CSW 0% CDS	5% CSW 5% CDS	10% CSW 10% CDS	15% CSW 15% CDS	SEM (N=4)	<i>P</i> linear	<i>P</i> Quad.
Final BW (kg)	117	117	114	115	1.31	0.096	0.825
Days to market	80.4 <sup>ab</sup>	79.5 <sup>a</sup>	83.5 <sup>ab</sup>	86.6 <sup>b</sup>	1.65	0.005	0.232
Feed intake (g/day)	2371	2383	2291	2269	51.66	0.086	0.738
BW gain (g/day)	1047 <sup>b</sup>	1055 <sup>b</sup>	966 <sup>ab</sup>	933 <sup>a</sup>	27.13	0.002	0.436
Feed:Gain	2.27 <sup>a</sup>	2.26 <sup>a</sup>	2.37 <sup>ab</sup>	2.43 <sup>b</sup>	0.03	0.000	0.300
Fat depth (mm)	22.4 <sup>b</sup>	18.2 <sup>a</sup>	19.2 <sup>a</sup>	18.2 <sup>a</sup>	0.82	0.001	0.037
Lean yield (%)	58.5 <sup>a</sup>	60.4 <sup>b</sup>	60.1 <sup>b</sup>	60.6 <sup>b</sup>	0.38	0.000	0.040
Minolta (L)	48.2	48.8	51.0	50.9	0.98	0.027	0.728
Drip loss (%)	9.1	8.4	10.3	10.8	0.73	0.043	0.415

a,b Values followed by different superscripts differ (P<.05).

**Take home message:** Increasing levels of both CDS and CSW to 10% or more of diet dry matter content compromised growth performance and some aspects of meat quality in growing-finishing pigs.

**Acknowledgement:** Financial support was provided by Ontario Pork, NSERC, OMAFRA and industry partners of the swine liquid feed association ([www.slfa.ca](http://www.slfa.ca)).

# Reducing the negative effect of feeding high potassium (K) containing co-products to pigs

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## Background:

With the high grain prices, alternative pig feed ingredients needed to be explored, including co-products from the bio-fuel and food industries. However, high dietary potassium (K) levels in co-product based diets contribute to reduced growth performance in pigs and abnormalities in kidney histology. The addition of  $K_2CO_3$  to the diet was previously shown to mimic the effect of feeding high levels of co-products (26% of feed DM) to pigs. This study was conducted to investigate the addition of calcium chloride ( $CaCl_2$ ) or sodium nitrate ( $NaNO_3$ ) to pig diets as means to overcome the negative effects of feeding 1.4% K containing diets.

## Methodology:

Yorkshire pigs from the University of Guelph herd were exposed to one of four dietary treatments (3 pens per treatment; 2 gilts and 2 barrows per pen; initial BW 22.8 kg  $\pm$ 0.5 kg). Pigs on the control treatment received a typical corn and soybean meal based diets (0.78% K). All other treatments were similar to the control with added 1.15%  $K_2CO_3$  to raise K levels to 1.4%. For diet  $K_2CO_3$ , no extra salts were added. For the diet  $NaNO_3$ , 0.40%  $NaNO_3$  was included. Finally, for diet  $CaCl_2$ , 0.76%  $CaCl_2$  was added at the expense of limestone to maintain calcium levels constant through out the treatments. Pigs were dry fed ad libitum and food consumption was recorded.

## Results:

Growth performance of growing pigs

	Dietary treatments				SE
	Control	+ $K_2CO_3$	+ $K_2CO_3$ + $NaNO_3$	+ $K_2CO_3$ + $CaCl_2$	
Feed intake (kg/d)	1.76	1.90	1.87	1.82	0.04
Gain (kg/d)	0.995	0.901	0.991	1.02	0.03
Feed (DM):Gain	1.77 <sup>ab</sup>	2.10 <sup>a</sup>	1.91 <sup>b</sup>	1.79 <sup>b</sup>	0.04

a,b Values followed by different superscripts differ ( $P < 0.05$ ).

Diet did not influence average daily feed intake or average daily gain, but diets  $CaCl_2$  and  $NaNO_3$  showed better feed:gain than diet  $K_2CO_3$  ( $P = 0.02$ ). No treatment effects were seen for carcass traits ( $P > 0.10$ ). External kidney damage was observed when pigs were fed diet  $K_2CO_3$ , and histology evaluations suggested internal damage predominantly on animals fed diet  $K_2CO_3$  and  $NaNO_3$ , but not  $CaCl_2$ .

## Implications:

$CaCl_2$  appears to alleviate effects of feeding high K to growing pigs without compromising kidney integrity; its relevance as an additive in high co-product diets is being investigated.

## Acknowledgement:

Technical support was provided by staff at Arkeil Research Station. Financial support was provided by Ontario Pork, Grand Valley Fortifiers and OMAFRA.

## Plasma prolactin and insulin concentrations in lactating sows following venous infusion of isoleucine, leucine, lysine, threonine, or valine.

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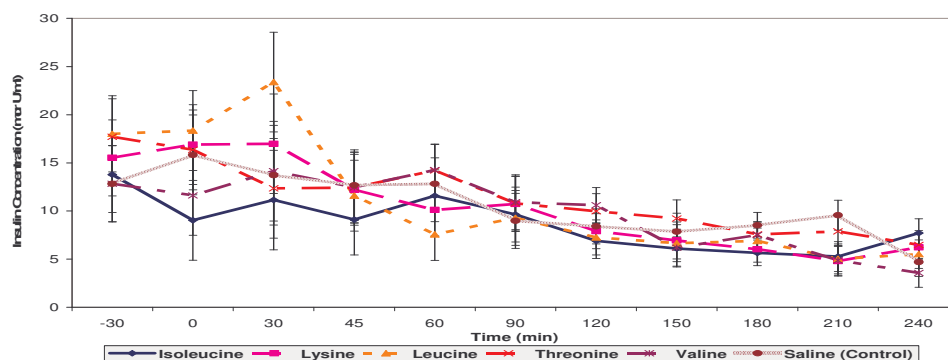
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**Background:** Understanding the relationship between amino acid (AA) supply and endocrine status during lactation may help in optimizing sow lactation diets. Supplying specific AA may alter the sow's endocrine status and, in turn, influence lactation performance. The objective of this study was to determine the short-term impact of infusing AA (isoleucine, leucine, lysine, threonine or valine) on the endocrine status of lactating sows.

**Methodology:** Multiparous Yorkshire sows (BW 253 ± 23kg), 7 to 10 days into lactation and fitted with ear vein catheters, were used in a 6 x 6 Latin square design. Sows were fed 3 times daily (1.5 kg at 600h and 1200h; 3.0 kg at 1830h) a corn and soybean meal based diet that contained 19.1% crude protein. One of the five amino acid solutions or saline (control) was infused at a rate of 33 ml/min during a 30 min period, 2 h after the 600h or 1200h feeding. The infused dose was equivalent to 18 ± 0.9% of true ileal digestible intake for each of the AA. Serial blood samples were taken, starting 30 min prior to infusion and until 240 min after infusion. Plasma concentrations of AA, insulin and prolactin were determined. Data were analyzed as repeated measurements using mixed model procedures.

**Results & conclusions:** Plasma amino acid levels, measured immediately after the end of the infusion period, increased between 3.1 times (valine) and 6.2 times (leucine) compared to those of controls ( $P < 0.05$ ). Plasma prolactin and insulin concentrations were not affected by amino acid infusion ( $P > 0.10$ ) Supplying 18% of daily amino acid intake intravenously for isoleucine, leucine, lysine, threonine or valine does not alter prolactin or insulin status of lactating sows.

Figure 1: Free Insulin Concentration in Blood Plasma. Values are LSM ± SEM



**Implications:** Milk production is one factor that impacts the productivity and survivability of piglets. In this study we could not establish a connection between plasma infusion of selected AA's and hormonal status of lactating sows.

**Acknowledgement:** Funding was provided by Ontario Pork and OMAFRA.

# Effects of Dietary Supplementation of Biacton™ on Growth Performance of Pigs from Weaning through Finishing Phases

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## BACKGROUND

Biacton™, a probiotic product of highly concentrated live microbial culture of the beneficent bacterium *Lactobacilli farciminis*. *L. farciminis*, is manufactured in France as an alternative probiotic growth promoting product.

## OBJECTIVES

The objective of this study was to examine and compare the efficacy of dietary supplementation (0.1-0.2%) of Biacton™ and a sub-therapeutical level of antibiotics, i.e., Lincomix-44 for weanling pigs and Tylan-10 for growing-finishing pigs, in improving growth performance, and carcass quality.

## METHODOLOGY

A total of 180 purebred Yorkshire pigs, with an average BW of 5.6±0.1 kg were weaned at 16±2 d of age in five periods, each consisting of 36 pigs, were used. The objective of this study was to examine and compare the efficacy of dietary supplementation (0.1-0.2%) of Biacton™ and a sub-therapeutical level of antibiotics, i.e., Lincomix-44 for weanling pigs and Tylan-10 for growing-finishing pigs, in improving growth performance, and carcass quality.

## RESULTS

Dietary supplementation (0.2%) of Biacton™ was only effective ( $P<0.05$ ) for improving growth rate and feed intake in weaning phase (5-10 kg) equivalent to the efficacy of a sub-therapeutical level of the antibiotics. Biacton™ supplementation had no effects ( $P>0.05$ ) on growth performance, including growth rate, feed intake and feed utilization in post-weaned (10-20 kg), growing (20-50 kg) and finishing (50-110 kg) phases. Whereas the growth-promoting effects of a sub-therapeutical level of antibiotics were consistently observed ( $P<0.05$ ) over these phases. Dietary supplementation of both Biacton™ and a sub-therapeutical level of antibiotics had no effects ( $P>0.05$ ) on major carcass quality parameters such as hot carcass weight, dressing percentage, backfat thickness and carcass lean in pigs.

## BENEFITS TO SWINE INDUSTRY

Dietary supplementation (0.2%) of Biacton™ may improve growth performance for weanling pigs in the absence of antibiotics.

## ACKNOWLEDGEMENTS:

Supported by OMAFRA-University of Guelph Partnership Program.

# Early-weaning Up-regulates the Expression of Sucrase-isomaltase in the Jejunum of the Piglet

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## BACKGROUND

Sucrase-isomaltase is a small intestinal brush border disaccharidase enzyme complex that hydrolyses both sucrose and maltose from dietary sources.

## OBJECTIVES

The objectives of this study were to examine the responses of sucrase isomaltase (SIM) activity and protein abundances associated with the mucosal homogenate (H), intracellular soluble (S), and the apical membrane (M) fractions as well as SIM mRNA abundance and its regulation during early-weaning in comparison with suckling pigs.

## METHODOLOGY

A total of 20 Yorkshire piglets, 10 suckling (SU) and 10 early-weaning (WN) with an average BW of 3 kg at the age of 10 d, were used in this study. Weanling piglets were fed a corn and soybean meal-based diet for 12 d. Proximal jejunal samples from both SU and WN groups were collected. Sucrose (0-25 mM) and maltose (0-60mM) was used in the enzymatic kinetic experiments. Abundances of SIM protein and mRNA were analyzed by Western blot and quantitative real time RT-PCR respectively, using  $\beta$ -actin as a control.

## RESULTS

The jejunal SIM maximal specific activity ( $\mu\text{mol}/\text{mg protein}\cdot\text{min}$ ) for sucrase and maltase increased ( $P<0.05$ ) in weaning piglets. Corresponding increases ( $P<0.05$ ) in the SIM protein abundance (arbitrary units) for the WN group was also observed in all jejunal fractions (H–34%, S–84% and M–61%) respectively. Furthermore, early weaning increased ( $P < 0.05$ ) the relative abundance of SIM mRNA by 1.9 fold (WN,  $0.306\pm 0.03$  vs. SU,  $0.105\pm 0.01$ ).

## BENEFITS TO SWINE INDUSTRY

The mechanism of transcriptional and post translational up-regulation of SIM expression is indicative of a greater digestive capacity and capability of sucrose and maltose in the early-weaned piglet. This allows for the inclusion of more cost-effective ingredients containing malted-starches and sucrose as a dietary source in the diets of weaning piglets.

## ACKNOWLEDGEMENTS

Final support for this research has been supported by Ontario Ministry of Agriculture Food and Rural Affairs (OMAFRA)-University of Guelph Partnership Program and the Natural Sciences Engineering Research Council (NSERC) of Canada.

# Effects of Dietary Levels of Tylosin on Growth Performance and Efficiency of Nutrient Utilization in Growing Pigs

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## BACKGROUND

Tylan<sup>TM</sup> has been approved by Canadian federal agencies for its application in swine industry for therapeutic and preventive purpose.

## OBJECTIVES

The objectives of this study were to examine effects of graded dietary levels of tylosin on growth performance and efficiency of utilization of dietary nutrients, including CP, calcium (Ca), and phosphorus (P) in growing pigs.

## METHODOLOGY

Sixty Yorkshire barrows, with an average initial and final BW of 31 and 48 kg, were fed four diets for 15 blocks according to a randomized complete block design. The four diets were corn and SBM-based and formulated to contain four levels of tylosin at 0, 11, 22, and 44 ppm, respectively. Each experimental block consisted of a 12-d pre-adaptation to the control diet, 9-d adaptation and a 5-d collection of total urine excretion, and fecal samples.

## RESULTS

Tylosin supplementation improved ADG ( $P<0.05$ ) at 11 ppm but not at the higher levels. Tylosin did not affect ( $P>0.05$ ) feed intake and feed conversion efficiency. Tylosin supplementation did not affect ( $P>0.05$ ) fecal content (mg H<sub>2</sub>S/g DM) of total volatile sulfides. The apparent DM digestibility values in the diets were not affected ( $P>0.05$ ) by tylosin. Tylosin improved ( $P<0.05$ ) the apparent CP digestibility at 11 ppm, but not at higher levels, yet did not affect ( $P>0.05$ ) the efficiency of CP retention at any of the tested levels. Efficiency of Ca utilization was not improved ( $P<0.05$ ) by tylosin. Dietary supplementation of tylosin had cubic effects ( $P<0.05$ ) on the efficiency of P utilization at the digestive and post-absorptive levels.

## BENEFITS TO SWINE INDUSTRY

Dietary supplementation of tylosin at 11 ppm may improve growth and efficiency of P utilization in growing pigs.

## ACKNOWLEDGEMENTS

Financial support for this research has been provided by Elanco Animal Health Canada, Inc., and Ontario Ministry of Agriculture Food and Rural Affairs (OMAFRA)-University of Guelph Partnership Program.

# Early-weaning Reduces the Small Intestinal Alkaline Phosphatase Activity at the Transcriptional and Post-transcriptional Levels

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## BACKGROUND

Expression of the small intestinal alkaline phosphatase (IAP) is enterocyte-differentiation dependent and plays essential roles in detoxification of pathogenic bacterial lipopolysaccharide endotoxin, maintaining luminal pH, organic phosphate digestion and fat absorption.

## OBJECTIVES

This study was conducted to examine the effect of early-weaning on changes in IAP digestive capacity and IAP gene expression in comparison with the suckling counterpart in the pig at the ages of 10-22 d.

## METHODOLOGY

A total of 20 Yorkshire piglets, 10 suckling (SU) and 10 early-weaning (WN) with an average BW of 3 kg at the age of 10 d, were used in this study. Weanling piglets were fed a corn and soybean meal-based diet for 12 d. Proximal jejunal samples from both SU and WN groups were collected and compared after *in vitro* enzymatic kinetic experiments. *P*-Nitrophenyl phosphate (0-10 mM) was used in the incubations. Additionally quantitative real time RT-PCR was performed on intestinal tissues using the Quantitect SYBR Green RT-PCR kit (Qiagen Inc., ON, Canada) via a Smart Cycler (Cepheid, CA), using  $\beta$ -actin as a control.

## RESULTS

Weaning decreased ( $P < 0.05$ ) IAP enzyme affinity by 26% and IAP maximal enzyme activity by 22% primarily in the jejunal region with the jejunum expressing 84-86% of the whole gut mucosal IAP digestive capacity. The majority (98%) of the jejunal mucosal IAP maximal activity was associated with the apical membrane with the rest (2%) as the intracellular soluble fraction of IAP. Weaning reduced ( $P < 0.05$ ) the abundance of the 60-kDa IAP protein associated with the proximal jejunal apical membrane by 64%. Furthermore, weaning reduced ( $P < 0.05$ ) relative abundance of the proximal jejunal IAP mRNA by 58% and this was associated with decreases ( $P < 0.05$ ) in the abundances of cytoplasmic (by 27%) and nuclear (by 29%) origins of IAP caudal-related homeodomain intestinal transcription factor *cdx1*.

## BENEFITS TO SWINE INDUSTRY

The decrease in the small intestinal IAP digestive capacity and the IAP gene expression at both the transcriptional and post-transcriptional levels during early-weaning in part accounts the susceptibility to post weaning diarrhea. Development of alkaline phosphatase supplements for the swine industry may offer an antibiotic free means of addressing post weaning diarrhea, decreasing the occurrence of enteric diseases and improving the associated growth-check.

## ACKNOWLEDGEMENTS:

Final support for this research has been supported by Ontario Ministry of Agriculture Food and Rural Affairs (OMAFRA)-University of Guelph Partnership Program and the Natural Sciences Engineering Research Council (NSERC) of Canada.

## EFFECTS OF MICROBIAL PHYTASE ON PHOSPHORUS CONTENT IN BONE AND SOFT TISSUES OF GROWING-FINISHING PIGS.

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### BACKGROUND

Phytase dose-related responses in pigs have been shown to vary due to phytase activity differences among categories of swine, type of phytase, intrinsic phytase present in the diet, Ca:P ratio and P level supplied by the diet. Therefore, studies into the effects of phytase level upon P presence in bone and tissues would help understand how different doses of phytase given to growing pigs affect P utilization.

### METHOD

Twelve castrated crossbred barrows (29.6 kg BW) were placed in metabolism cages for balance studies and then sacrificed for P determination in bone and soft tissues (liver, heart, kidney and muscle). Treatments consisted of a standard feed containing 0.17% of available P, and increasing levels of phytase (produced by fungi of the *Aspergillus niger* group) corresponding to 253, 759, 1265 and 1748 PU/kg feed, denoted by T1, T2, T3 and T4, respectively.

### RESULTS

The values of P (g/kg DM) in muscle and heart were statistically higher for T4 and tended to be higher in liver and kidney for this treatment as well (Table 1). Values of P in bone (g/ DM) were statistically higher for T2 and similar between T1, T3 and T4.

Table 1. Phosphorus content of bone and soft tissues (g/kg DM)

Tissues	Phytase, PU/kg of feed				SEM
	T1	T2	T3	T4	
Bone	76.47 <sup>x</sup>	85.03 <sup>y</sup>	70.49 <sup>x</sup>	70.01 <sup>x</sup>	2.26
Liver	8.97	5.75	7.95	9.94	1.57
Muscle	6.41 <sup>xy</sup>	4.70 <sup>y</sup>	5.70 <sup>y</sup>	10.39 <sup>x</sup>	0.42
Kidney	9.70	6.02	6.95	11.19	1.37
Heart	7.40 <sup>xy</sup>	5.49 <sup>xy</sup>	4.30 <sup>y</sup>	9.34 <sup>x</sup>	1.88

<sup>x,y</sup> Means within row and treatment category with different superscripts differ ( $P < 0.05$ ).

### BENEFITS TO THE SWINE INDUSTRY

Phosphates generated by a high phytase level in the diet are mainly channeled to soft tissues therefore addressing metabolic needs such as growth.

### ACKNOWLEDGEMENTS

This project was in part supported by the Canada Research Chairs Program, FAPESP and OMAFRA.

# Meta-analytic study of phosphorus excretion in pigs

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## BACKGROUND

Many studies have been carried out to better understand the factors affecting P excretion in pigs in an attempt to search for more efficient ways of minimizing the detrimental effects of P on the environment. The large amount of data available allows researchers to use meta-analysis as a tool to synthesize results from several studies.

## METHOD

A meta-analysis was performed using 190 data points from 69 studies to determine the most important dietary features affecting P excretion in pigs. In a second analysis, a subset of the data (total 27 studies) containing only values of P excreted in feces of animals that were fed non-phytase was examined. The model found was evaluated using data from another 27 studies.

## RESULTS

The following model was generated from the first meta-analysis:  $P \text{ feces (g/(kg BW}^{0.75} \times d))} = -0.00002 \text{ phytase} \times \text{phytate} + 0.33 P - 0.05 \text{ Ca/P} + 0.14$ , with all parameters significant. The following linear model was generated in the second analysis:  $P \text{ feces (g/(kg BW}^{0.75} \times d))} = 0.4 P \text{ intake} + 0.07$  (Figure 1). The predicted values were strongly related to measured values.

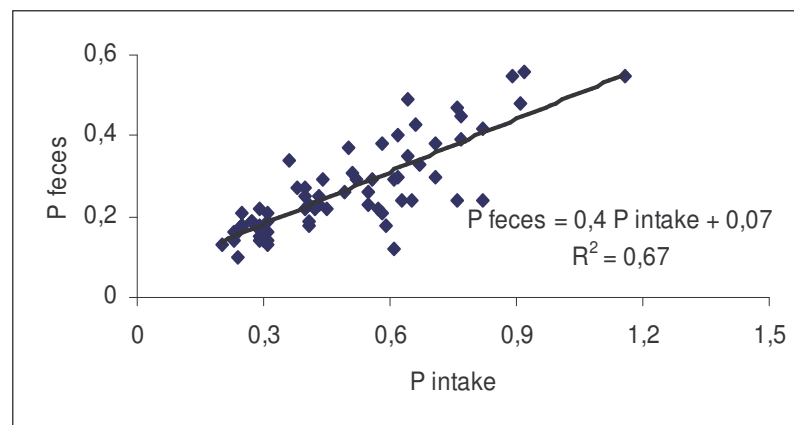


Figure 1. P excreted in feces vs. P intake ( $\text{g}/(\text{kg BW}^{0.75} \times \text{d})$ )

## BENEFITS TO THE SWINE INDUSTRY

According to the first model P, Ca/P and the interaction between phytate and phytase present in the diet are the main factors to be considered when producers aim to reduce P excretion in pigs. Moreover the linear model presented may be used to predict P excretion in pigs when ingested P is known and no phytase is added to diet.

## ACKNOWLEDGEMENT

This project was supported by the Canada Research Chairs Program.

## Predicting phosphorus retention in post-weaned pigs

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### BACKGROUND

Phosphorus retention in pigs is related to P utilization which depends on the metabolic processes that the animal is undergoing. Moreover monogastrics have a very low capability to hydrolyze phytate into phosphate which may also affect P retention. Knowledge of P retention is important to further understand P utilization by different categories of animal.

### METHOD

A database on P balance was built using 376 observations from 23 experiments with pigs weighing between 6 and 17 kg, and P retention ( $\text{g}/(\text{kg BW}^{0.75} \times \text{d})$ ) was plotted against total P intake ( $\text{g}/(\text{kg BW}^{0.75} \times \text{d})$ ). The parameters found by Schulin-Zeuthen et al. (*J. Anim. Sci.*, 2007, 85:1953-1961) for the functions: straight line (SL), monomolecular (M), Gompertz (G) and Richards (R) were then used to predict P retention values for the database (treated as a single dataset), and the functions were further evaluated. Observed values of P retention were compared to the predicted values using means square prediction error (MSPE) and concordance correlation coefficient (CCC).

### RESULTS

The MSPE values were 0.034, 0.119, 0.005 and 0.029 for SL, M, G and R respectively. The accuracy (Cb) and precision ( $\rho$ ) values obtained from decomposition of CCC are presented in Table 1. The MSPE method showed that G gave the best prediction for P retention whereas the CCC method suggested that M gave the best prediction, although G also predicted well.

### BENEFITS TO THE SWINE INDUSTRY

Overall the Gompertz equation gave the best fit and may be used to predict P retention in post-weaned pigs fed no phytase.

**Table 1.** Comparison of observed and predicted values of P retention using concordance correlation coefficient (CCC) decomposed into accuracy (Cb) and precision ( $\rho$ ).

Functions	Symbols	Observed mean	Predicted mean	Cb	$\rho$
Straight line	SL	0.26	0.20	0.85	0.95
Monomolecular	M	0.26	0.51	0.91	0.97
Gompertz	G	0.26	0.25	0.87	0.95
Richards	R	0.26	0.20	0.89	0.95

### ACKNOWLEDGEMENT

This project was supported by the Canada Research Chairs Program.

# Effects of feeding level, dietary protein level and gender on pig growth performance and meat quality

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## Background

Most on-farm feeding systems try to maximize feed intake during the growing and finishing periods. However, recent research at the University of Guelph (McEwen and Mandell, 2008) indicated that feed efficiency could be improved if pigs were limit fed during the growing period followed by *ad libitum* feeding until market. Since feed costs are presently increasing, a limit feeding approach for both energy and protein may be a means to further reduce expenditures in a market where carcass returns do not keep pace with costs of production.

## Methodology

Ninety-four feeder ( $37.7 \pm 5.3$  kg) pigs were assigned to a 2 x 2 x 2 factorial design trial with 2 dietary protein levels (0.9 versus 0.63% lysine), 2 feeding levels (*ad libitum* vs. 70% of *ad libitum*) in the growing period and 2 genders (barrows and gilts) represented. At 65 kg body weight (BW) all pigs were *ad libitum* fed until slaughtered at approximately 115 kg BW.

## Results

Overall body weight (BW) gains were greater ( $P < 0.001$ ) for FF than LF even though LF pigs exhibited ( $P < 0.001$ ) compensatory gain during the finishing phase. BW gains were greater ( $P < 0.001$ ) for barrows than gilts while no protein effect ( $P > 0.05$ ) was observed.

Overall feed intakes ( $\text{kg.pig}^{-1}$ ) and efficiencies (F/G) were similar ( $P > 0.10$ ) across treatments most likely due to time on feed differences ( $P < 0.0001$ ) for FF vs. LF pigs.

Dressing percentage was 0.8 units greater ( $P < 0.01$ ) for FF versus LF pigs, with no protein and gender effects ( $P > 0.35$ ).

Backfat depth was greater ( $P < 0.01$ ) for barrows, with larger ( $P < 0.001$ ) loin eye areas for gilts. Feeding and protein levels did not influence ( $P > 0.10$ ) backfat depth or loin eye area.

Loin meat quality measures (color, marbling, Warner-Bratzler shear force, pH at 24 h, drip and cooking losses) were not influenced ( $P > 0.05$ ) by feeding and protein levels or gender.

## Benefits to the Swine Industry

Results contradict McEwen and Mandell (2008) where limit feeding during the growing phase improved F/G but reduced marbling. While Therkildsen et al. (2002) reported improvements in tenderness with limit feeding, meat tenderness was not affected by limit feeding in the present study or in McEwen and Mandell (2008).

## Acknowledgments

The authors would like to thank Ontario Pork and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) for their support. Data collection and animal care by staff in the Ridgetown Swine Research Facility were also greatly appreciated.

# The Effects of Limit Feeding and Gender on Pig Growth Performance, Carcass and Meat Quality.

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## Background

Therkildsen et al. (2002) observed improved feed efficiency, meat quality and tenderness when pigs were limit fed during the growing period (restricted access to feed) in a Danish production system that markets pigs at lighter body weights (BW) than the Ontario swine industry. This trial investigated how limit feeding during the growing period would impact growth performance, carcass and meat quality in Ontario pork production.

## Methods

One hundred and five feeder pigs ( $30.3 \pm 2.8$  kg) were randomly assigned to pens by gender and then to one of three feeding strategies:

Control (full fed: FF) : - A corn/soybean meal based diet ((18.5% CP; 0.85% lysine) was fed *ad libitum* until the pigs (within pen) attained 60 kg body weight (BW). Pigs were then switched to a 15% CP (0.7% lysine) finisher diet until marketed at 110 kg BW.

Limit Fed-Low Lysine (LF-LL): - Feed allocations were limited to 70% of *ad libitum* feed intake by the Control group until the pigs weighed 60 kg; the pigs were then fed the Control finisher diet (18.5% CP; 0.85% lysine) *ad libitum* until marketed at 110 kg BW.

Limit Fed-High Lysine (LF-HL): - Feed allocations were limited to 70% of *ad libitum* feed intake by the Control group until the pigs weigh 60 kg; the pigs were then fed a higher lysine finisher diet (15.5% CP; 1% lysine) *ad libitum* until market weight.

## Results

Overall daily gains (kg/d) were increased ( $P < 0.001$ ) for FF versus LF (LF-LL, LF-HL) pigs even though compensatory gains in the LF pigs increased ( $P < 0.0001$ ) gains versus FF pigs during the finishing period. The higher gains overall for FF pigs decreased ( $P < 0.0001$ ) days to market versus LF pigs.

Lower ( $P < 0.05$ ) F/G values for LF pigs in the finishing period resulted in decreased ( $P < 0.01$ ) overall F/G versus FF pigs (kg feed/kg gain: FF-2.68, LF-LL-2.49, LF-HL-2.51; SE=0.04).

There were no differences ( $P > 0.05$ ) between feeding strategies in meat quality (pH, loin colour, drip loss, and Warner-Bratzler shear force). In contrast, marbling was reduced ( $P < 0.05$ ) for LF-HL pigs versus FF and LF-LL pigs.

## Benefits to the Swine Industry

The results indicated that limit feeding during the growing phase may improve overall feed efficiency to market weight while not adversely affecting most carcass or meat quality parameters.

## Acknowledgments

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## Use of phytase in sow diets.

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### Background:

Phytase has been shown to increase calcium and phosphorus digestibility in sows. Besides rendering much phosphorus unavailable, phytate also binds many trace minerals. Some researchers were able to entirely remove trace mineral supplementation and maintain performance and quality of market hogs with phytase supplementation. The objectives of this study were to determine the effect of phytase supplementation, with or without trace mineral supplementation, on sow and piglet performance and mineral digestibility for sows during one lactation.

### Methodology:

Thirty-six sows were fed one of four treatments in a 2 x 2 factorial design. One factor was mineral supplementation: Two treatments received a commercial mineral supplement (+M) and two treatments received reduced calcium and phosphorus and no trace mineral supplement (-M). The other factor was phytase supplementation: Two treatments received phytase supplementation (+P) and two treatments did not receive phytase supplementation (-P). Sow and piglet performance was recorded. Chromic oxide was used as an indigestible marker. Manure samples were taken on day 28 so that apparent fecal digestibility could be determined.

### Results:

There was no effect of treatment on sows (weight, back fat, milk components) or piglet (pre-weaned mortality, weaning weight) performance. In contrast to other work that has been done, there was no effect of treatment on apparent fecal digestibility of minerals. There was, however, an effect of mineral supplementation on sow feed intake with the reduced supplementation sows consuming more feed. Total tract mineral disappearance (defined as: average sow feed intake over days 26 – 28 x the mineral concentration in the feed x the apparent fecal digestibility of the mineral) was not affected by treatment. However, it is interesting to note that the numerical responses to apparent fecal digestibility were opposite to the numerical responses for the total tract mineral disappearance for every mineral.

### Implications:

Data analysis is ongoing but it appears that, at least in the short term, sows do not require the current typical levels of calcium and phosphorus and any trace mineral supplementation they receive. The long term effect of reduced mineral supplementation was not investigated and could conceivably alter such conclusions. While definite conclusions cannot be made, these data also suggest that current levels of calcium, phosphorus and trace mineral supplementation are negatively impacting sow feed intake.

### Acknowledgements:

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# **Manipulating the Uterus as a Means to Produce Uniform and Large Litters**

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## **BACKGROUND**

Prenatal mortality upto 30% before gestation day (gd) 30 and additional 10-15% around gd50-80 is a prime concern to the commercial swine industry in North America. Poor blood supply to the developing conceptuses is postulated to be a leading cause. Small scale studies have shown that porcine fertility can be promoted immunologically through injection of seminal plasma, growth factors, or immunomodulators. We have identified an immune stimulant, manufactured currently for human use as effective in porcine fecundity enhancement. We posit that immune-stimulant will enhance immune cell-mediated porcine endometrial angiogenesis and thereby fetal survival, reducing heterogeneity between littermates. Our preliminary results show multiple beneficial effects including increase in litter size and reduction in the numbers of runts in the litters born to treated animals.

## **METHODOLOGY**

For molecular profiling of endometrial lymphocytes and dendritic cells, we have utilized laser capture microdissection to collect these cell types from the frozen endometrial sections. Gene expression for important angiogenic genes (VEGF family) and cytokines was studied using real time PCR. Comparisons of gene expression between endometrial lymphocytes, dendritic cells were done with endometrial biopsies and trophoblasts obtained from healthy and arresting conceptus attachment sites. Preliminary clinical trial utilizing proprietary immune stimulant was conducted at the Arkell swine research station. The proprietary immune stimulant was introduced via intra-uterine infusion (single dose) just prior to artificial insemination. Fetal development in treated and control animals was tracked using ultrasound examination.

## **RESULTS**

Endometrial lymphocytes, dendritic cells and trophoblasts expressed all VEGF members but relative transcription varied with gestational age and implantation site viability.

Preliminary results from clinical trial conducted with the proprietary immune-stimulant demonstrated stimulation of maternal and fetal angiogenesis

This resulted in the increase in litter size and reduction of runts in treated compared to placebo animals

## **BENEFIT TO THE SWINE INDUSTRY**

We have made an excellent progress in advancing knowledge on the immunological aspects of spontaneous fetal loss during porcine pregnancy. In our preliminary therapeutic trial, we showed litter size can be stimulated and numbers of runts/litter reduced. We are currently working on a recent formulation specifically designed for use in livestock species.

## **ACKNOWLEDGEMENTS**

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## **Swine AI research: 20+ years playing with sperm**

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### **Background:**

Twenty years ago, less than 5% of Ontario's pigs were bred by artificial insemination (AI), and their owners were largely purebred breeders using purebred semen from the single Ontario supplier. Today over 80% of all Ontario's pigs are bred by AI using purebred, crossbred and pooled semen obtained from a multitude of commercial semen providers or their own in-house boar battery. Many folks in southern Ontario contributed in building this sometimes-rocky road to AI success, which despite detours, switchbacks and roundabouts, has got us effectively using this major tool supporting genetic diversity, herd health and rapid genetic improvement (particularly in growth rate and days-to-market), not just in our province but worldwide.

### **Methodology:**

Where possible, trials in semen quantity and quality use multiple ejaculates from one boar, because each ejaculate can be different, and then include many boars, because, of course, individual males are different. How a boar is housed, fed and handled can affect semen in different ways. The effect of storage on sperm quality is best tested on an ejaculate immediately after collection, and then tested again during and after storage. Humans and machines can assess the % of sperm that are moving, but machines are more consistent! The % of living sperm is tested separately because a sperm might be alive but not swimming. We are trying to find genetic factors that might predict fertility, so that a producer can include that important trait when selecting boars. We can do a variety of laboratory tests that approximate sperm's ability to fertilise, but only breeding a female and counting piglets really tests the bottom line.

### **Results:**

Boars and ejaculates can differ significantly in many ways. A typical Ontario heat wave first affects semen quality days after the heat wave ends, and can cause temporary or permanent damage. Different extenders used to store liquid boar sperm are effective for very different periods of time. We still can't freeze boar semen effectively enough for widespread commercial success. A sperm has to do many things in the proper way at the proper time to fertilise, and while we are learning more and more about those, many details are not yet understood.

### **Conclusions:**

AI is an important tool now engrained in Ontario swine production. Many factors affecting semen quality and quantity are becoming known. We need to control housing conditions particularly health, welfare and temperature of the boars to ensure high quality semen. The exact chemical reactions that sperm use to prepare to fertilise an egg are becoming better understood and may lead to better fertility prediction.

### **Benefits to the swine industry:**

AI contributes greatly to efficient, effective swine production and readily supports breeding for pigs to supply unique products desired by specific markets. More effective semen storage will further enhance those benefits and allow an international genetics market to develop.

# Reactive Oxygen Species and Boar Sperm Function

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## Background:

Boar sperm are very sensitive to damage, which makes it difficult for them to survive and fertilise after normal storage for artificial insemination as liquid semen, and particularly more extreme conditions involved in freezing and thawing. Boar sperm are very susceptible to Reactive Oxygen Species (ROS) because of their natural high content of polyunsaturated fatty acids. ROS are very dynamic chemicals that can quickly damage the cells that create them, particularly by damaging their membranes. We compared frozen-thawed boar sperm to fresh sperm we exposed to Xanthine/Xanthine Oxidase (XA/XO) which generate ROS, measuring how much ROS they contained, as well as damage to their important fertilizing membranes (indicated by lipid peroxidation and/or phospholipase A (PLA) activity), and their more obvious functional needs to be alive, motile and able to fertilise (acrosome react; AR)

## Methodology:

Multiple ejaculates from different boars were used to measure intracellular levels of two important ROS ( $O_2^{\cdot-}$  and hydrogen peroxide), as well as lipid peroxidation and PLA activity in living and dead sperm by flow cytometry. Motility was measured by computer assisted analysis, and AR by microscope after 0 and 4 hours in a simple BTS buffer or a capacitating media that should encourage AR and fertilization.

## Results:

Exposing fresh boar sperm to the ROS generating system rapidly increased  $H_2O_2$  and lipid peroxidation in all sperm, increased PLA levels in dead sperm, and had no effect on sperm intracellular production of  $O_2^{\cdot-}$ . Sperm simultaneously became immotile, but were alive and many more sperm underwent the acrosome reaction in capacitating incubations. Frozen-thawed sperm are less motile, many are dead, and their internal levels of  $O_2^{\cdot-}$  decline; their hydrogen peroxide levels don't change.

## Conclusions:

Fresh boar sperm generate hydrogen peroxide when exposed to an ROS generator, but freezing and thawing causes different ROS to decline. Boar sperm motility, acrosome integrity and lipid peroxidation are also very sensitive to both ROS and freezing and thawing, but in different ways. ROS may cause boar sperm to prematurely acrosome react.

## Benefits to the swine industry:

The current processes for freezing and thawing sperm for AI does change sperm ROS content, and understanding how ROS regulate sperm preparation for fertilization should help us optimize boar sperm preservation for AI.

## Acknowledgements

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## **Na<sup>+</sup>K<sup>+</sup>-ATPase and the effect of ouabain in boar spermatozoa**

**Meghan Mackenzie Bell and Mary M. Buhr**

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Spermatozoa must complete capacitation to be able to fertilize an oocyte. We have shown that the transmembrane protein Na<sup>+</sup>K<sup>+</sup>-ATPase, in addition to its traditional enzyme function, acts as a signalling molecule, and the steroid hormone ouabain, a highly specific ligand for Na<sup>+</sup>K<sup>+</sup>-ATPase, induces capacitation in bull sperm. Na<sup>+</sup>K<sup>+</sup>-ATPase contains alpha and beta subunits, whose isoforms have been localized in porcine spermatozoa. The objective was to explore Na<sup>+</sup>K<sup>+</sup>-ATPase's role in porcine capacitation.

A minimum of 4 fresh ejaculates from mature boars of proven fertility was used per experiment. Ouabain's interaction with Na<sup>+</sup>K<sup>+</sup>-ATPase subunits was assessed by incubating sperm with 0 - 500µM ouabain. Binding of alpha isoforms 1, 2, 3 and beta 2, 3 were determined by immunofluorescence after 10 minutes. Ouabain's effects on capacitation were assessed microscopically on sperm incubated for 0, 4 or 6 hours in capacitating conditions with 0, 50 or 100µM ouabain.

Ouabain lowered the visual fluorescence intensity of alpha subunits 1 and 3, and did not affect the detected presence of the isoforms alpha 2 or beta 1-3. As expected, significantly more sperm underwent an ionophore-induced acrosome reaction (AR) after 6 hours of incubation in capacitation medium versus control (BTS). Ouabain increased the spontaneous (non-ionophore induced) AR in spermatozoa incubated in BTS, but not capacitating media, in a time dependent manner (0hr incubation, 15 vs. 24 ± 1.7%AR, mean ± SE, 0 vs 100 µM ouabain, respectively; P=0.01), compared to 6hr (33 vs 40 ± 1.67 %AR, 0 and 100 µM ouabain, P<0.0001). In capacitation media, although at time 0 the % AR at 0 µM ouabain was identical to the BTS treatments, ouabain caused a significant drop in %AR at 6 hours to 8 ± 1.7% AR (P<0.0001).

Na<sup>+</sup>K<sup>+</sup>-ATPase is operative in boar sperm capacitation, with its specific ligand ouabain interacting preferentially with the alpha 1 and/or 3 isoforms to influence the onset of capacitation in porcine spermatozoa.

## TEETHING AND FEEDING BEHAVIOUR IN YOUNG PIGLETS

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### BACKGROUND

Performance and health of newly weaned piglets often hinge on their ability to consume feed quickly after weaning, yet most piglets still consume insufficient food and experience weight loss at this time. As that the majority of a piglet's pre-weaning nutrition is derived via suckling motor patterns and not chewing, we wanted to examine how dentition influences feeding in young piglets. The objectives of this study were to determine if premolar eruption or occlusion (i.e. the contact of premolars in opposing jaws) were associated with creep feeding behaviour or creep feed intake in piglets under 28 days of age.

### METHODOLOGY

Twenty-four litters of Yorkshire piglets (N=233) were given dental exams at ages 2, 6, 9, 13, 16, 20, 23, and 27 days. On day 5, piglets were provided with creep feed that was mixed at 1% inclusion with chromic oxide (Cr<sub>2</sub>O<sub>3</sub>). Fecal samples were obtained from each piglet on the same days as dental exams and were visually assessed for the presence of Cr<sub>2</sub>O<sub>3</sub>, indicating prior ingestion of feed. The duration of time piglets spent with their head in the creep feeder and the number of feeder visits were determined from continuous video recordings for 6 hr/day (07:00-10:00, 13:00-16:00) on days 7, 10, 14, 17, 21, and 24. Birth and weekly weights were recorded, as were sow parity and age, number of live born piglets, number of stillbirths, number of mummies, and male:female ratio of the litter.

### RESULTS

Piglets 17 days of age and younger with their premolars erupted and occluded spent less time at the feeder and visited it less frequently, possibly in response to the inflammation and gingival sensitivity caused by tooth eruption. However, piglets 21 days of age and older with their premolars erupted and occluded spent more time at the feeder and visited it more often, suggesting that by these ages piglets are more motivated to feed when having these dental conditions. Feed ingestion was not associated with dentition, though methodological problems may have occurred.

### BENEFITS TO THE SWINE INDUSTRY

This study is the first to identify dentition as a factor in the development of feeding behaviour in young piglets. As fundamental knowledge relating to dental development emerges, swine producers may be better able to employ management practices that best suit their herd.

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