

THE 10TH MIKE WILSON SWINE RESEARCH DAY

JUNE 14, 2013

9:30 AM – 3:30 PM

Arboretum Center, University of Guelph



The 10th Annual Mike Wilson Swine Research Day

June 14, 2013

9:30 AM – 3:15 PM

Arboretum Centre, University of Guelph

- 9:30 AM Registration and coffee
- 10:00 AM Bob Friendship: Welcome
- 10:05 AM Dr. John Harding, University of Saskatchewan: Solving reproductive PRRS
- 11:00 AM Kate Bottoms: Investigating potential disease risk associated with feed delivery
- 11:15 AM Ashley Roberts: Welfare farm audits
- 11:30 AM Marko Rudar: Dynamics of whole body nitrogen retention during an immune challenge
- 11:45 AM Mackenzie Slifierz: Molecular association between health and growth of pigs
- 12:00 Noon Lunch and posters
- 13:30 PM Janet MacInnes: Tonsil microbiome – possible roles in health and disease
- 13:45 PM Patrick Boerlin: *Haemophilus parasuis*
- 14:00 PM Wilfredo Mansilla: Nitrogen utilization in growing pigs
- 14:15 PM Phil McEwen: Glycerol as feed ingredient for growing pigs
- 14:30 PM Terri O'Sullivan: Peri-weaning failure to thrive syndrome update
- 14:45 PM Vahab Farzan: *Clostridium perfringens* type A – cause of piglet diarrhea?
- 15:00 PM Kees de Lange: Announcement of winning posters and closing remarks

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Preliminary results of the Canadian Pregnant Gilt Project: a multidisciplinary approach to help solve reproductive PRRS

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Controlling the reproductive and congenital effects of PRRSv is vitally important for reducing the impact of the disease in North America. Until we succeed at this, it will be very difficult to make sustained progress at the farm or regional level. Reproductive PRRS however, is proportionately understudied, particularly in controlled conditions, because large-scale studies require immense resources. At present, the mechanisms of PRRSv induced fetal infection, morbidity and death are not understood, nor are the underlying genes controlling these traits. Fetal death is unlikely caused by viral replication in fetal tissues, but rather mechanisms occurring in the pregnant uterus and placenta in response to the virus.

Controlled experimental studies enable the discovery of genes and mechanisms that can be subsequently investigated in applied field trials. The tissues and data we've collected since 2011 during our PRRS Pregnant Gilt Project represent a huge opportunity to understand fetal PRRS and maternal transmission of the virus, and ultimately aims to deliver tools to control reproductive PRRS. The PRRS Host Genetics Consortium (PHGC) has used genomic approaches to deliver tools to reduce the impact of PRRSv in nursery pigs. This led to the identification of a region on swine chromosome 4 (SSC4) that encodes reduced susceptibility to PRRSv infection in growing pigs. As part of our ongoing work we will determine if the favorable SSC4 allele, associated with decreased viral load and increased ADG in the PRRS nursery pig model, is applicable for reproductive PRRS. However, we anticipate that different mechanisms apply to pregnant females and fetuses, and therefore, will also be examining a full spectrum of genes in both parents and offspring.

The Pregnant Gilt Project is a sub-activity of a Genome Canada sponsored large scale project investigating the application of genomics to improve swine health and welfare. It is multidisciplinary and multi-institutional, spearheaded by Dr. Graham Plastow, University of Alberta. The collaborative partnership includes the swine industry represented by PigGen Canada (a research consortium of nine pig breeding organizations that represent 95 percent of pig genetics in Canada), funding agencies including Genome Canada, Genome Alberta, Genome Prairie, CSHB, ALMA, USDA, NPB, and researchers at the Universities of Alberta, Saskatchewan, Calgary, Guelph, Iowa State, Kansas State, Lincoln-Nebraska, Roslin and the Beltsville Agricultural Research Centre (BARC) USDA. The project complements and builds on the activities of the PHGC initiated in 2007 by Drs. Joan Lunney, Jim Reecy and Bob Rowland, to identify genes and host responses associated with resistance to PRRSv infection and related growth effects in nursery pigs. The project is in year 2 of its three-year mandate to deliver genetic tools to assist in the selection of PRRSv and PCV2 resilience replacement animals.

The specific objectives of the pregnant gilt project are to: 1) deeply characterize the phenotypic responses to late gestation PRRSv infection in pregnant gilts, 2) determine phenotypic and genotypic

traits that are associated with resilience to PRRSv, and 3) determine if PRRS severity is epigenetically programmed and associated with the low birth weight phenotype.

Briefly, purebred Landrace gilts, synchronized and bred homospermically to York boars, were infected with type 2 PRRSv (NVSL 97-7895) at gestation day 85 (n=114) or were sham-inoculated (CTRL; n=19). Clinical signs were monitored daily. Blood, collected on 0, 2, 6, 19 days post inoculation (dpi), provided samples for measurement of PRRSv RNA concentration using a strain-specific in-house qPCR, white blood cell counts, and lymphocyte subset typing (T-helper, CTL, B-cells, gamma-delta cells, NK cells, myeloid cells) using flow cytometry. Gene networks regulating the *in vitro* responsiveness of peripheral blood mononuclear cells (PBMC) to homologous PRRSv and phorbol myristate acetate/ionomycin (PMA/I) stimulation will be assessed. Gilt sera and the supernatants of PRRSv and PMA/I stimulated PBMCs will be tested for innate, regulatory, T helper 1 (Th1) and Th2 cytokine levels by fluorescent microsphere immunoassay (FMIA) and ELISA.

Following humane euthanasia at 21 dpi, gilts and fetuses were dissected. The position, preservation status and body weight of each fetus was recorded, as was the weight of both ovaries, and the number and weight of dissected corpus lutea. Fetal gonads were paraffin embedded allowing histological comparison of the progeny of high and low birth weight dams. Serum and multiple tissues are collected enabling measurement of PRRSv RNA concentration in gilt tissues (uterus, lung, tonsil, tracheobroncheal and reproductive LN), amniotic fluid, fetal sera and thymus. Alveolar macrophages harvested from gilt bronchoalveolar lavage fluid were tested to determine variability in functional responses and kinase activities associated with PRRSv infection (kinases have central roles in virtually all cellular behaviour, including immune defence). Pertinent fetal and maternal tissues, including the uterine and placental tissue at the stump of the umbilical cord are being examined histopathologically to assess mechanisms of fetal death and variability in host pathologic responses. Gilts, sires and fetuses will be genotyped with the Illumina Porcine SNP60 BeadChip, enabling genome wide association studies. Bayesian genomic selection models will be developed to evaluate associations among phenotypic and genotypic data using imputed or measured 60K data from gilts and fetuses (n=1000-1400 PRRSv infected gilts and fetuses).

To date, our team has completed the animal experiments, and have accumulated extensive laboratory data including virus levels in gilts and fetuses, changes in blood leukocyte subsets and cytokine proteins in dams following infection, proteolytic and kinomic analysis of alveolar macrophages, maternal and fetal pathology, and transcriptomic and peripheral blood mononuclear cell (PBMC) responses following re-stimulation with PRRSv and PMA/I. All dams and sires have been 60K SNP genotyped and sample prep to enable fetal genotyping is underway.

Two PRRSv-infected gilts aborted (17 and 20 dpi) and 1gilt died on 11 dpi. Apart from a reduced feed intake and rise in rectal temperature in some individual gilts, no other clinical signs were noted following PRRSv challenge. All infected gilts had quantifiable PRRSv levels on 2 dpi (mean $2.9 \pm 0.5 \log_{10}$ copies/ μ l). PRRSv RNA concentration in serum peaked at 6 dpi (mean $4.3 \pm 0.7 \log_{10}$ copies/ μ l) but demonstrated considerable variation between gilts. At 21 dpi, about 15% of gilts were able to clear the virus from the blood, ~41% had detectable but non-quantifiable PRRSv RNA levels in serum, and ~43% had quantifiable levels. PRRSv RNA levels were higher in lymphoid tissues than in sera or lung. No gilt cleared the virus from reproductive or tracheobronchial lymph nodes by 21 dpi.

Out of 1393 PRRSv challenged fetuses, $59 \pm 23\%$ survived until termination. The within litter survival rate ranged from 5% to 100%. Percent fetal survival was negatively associated with PRRSv RNA

concentration in reproductive lymph node ($P = 0.04$), but was not associated with PRRSv concentration in serum at 2, 6 or 21 dpi, average serum concentration, lung or tonsil. For each 1 log increase in viral load in reproductive lymph node, fetal survival decreased 5.4% in PRRSv infected litters terminated 21 dpi.

Fetal preservation was scored as viable (VIA), live meconium stained (MEC), decomposed (DEC), autolysed (AUT). MEC fetuses were mainly observed in infected gilts suggesting a PRRS-related pathologic process. PRRSv RNA was detected in over 90% of MEC, DEC and AUT, but in fewer VIA fetuses. In ~10% of fetuses, PRRSv was not detected in either serum or thymus, even though moderate to high PRRSv concentration was detected in the endometrium adjacent the umbilical attachment. Conversely, ~7% of autolysed and decomposed fetuses had no detectable levels of PRRSv in fetal thymus, possibly due to degradation of virus RNA within 21 days or death of the fetus prior to infection of the fetal thymus. Fetal preservation and PRRSv levels were highly variable among litters and suggest differences in PRRSv resistance at the fetal level. The most consistent microscopic lesion observed was endometrial inflammation and occasionally, early signs of placental separation. Lesions varied in severity and additional tests on these samples are underway to understand the underlying mechanisms and the role of PRRS virus particles, macrophages, and apoptosis in the pathogenesis of PRRS in the uterus and placenta. Our data and previous research support the notion that fetal death is independent of PRRSv replication in fetal tissue.

These and more results will be presented at the meeting. We wish to thank the many people assisting with this project, including barn, animal care and necropsy staff, students, technicians and administrative personnel.

Investigating disease risk associated with feed delivery

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BACKGROUND – Certain pathogens can be spread between farms via fomites, including contaminated boots, clothing, hands, and vehicles. Additionally, feed and/or specific protocols for the delivery of feed have been associated with the transmission of swine diseases. This project allowed for the identification of economically and logically feasible management changes at the feed company and farm level that will reduce the potential for disease transmission associated with the delivery of feed.

METHODOLOGY – Initially, discussions with swine producers and feed company personnel (managers, dispatchers, truck drivers) explored what current protocols are used in the industry to address the potential for disease spread via feed delivery, and to identify possible changes that could further reduce that risk. Next, a 6-week pilot study was conducted in order to collect information about what is currently happening in the industry, to determine the frequency of some of the identified issues in the day-to-day delivery of feed, and to trial the use of reusable rubber boots.

RESULTS – The pilot study included 3 feed companies, 40 feed truck drivers, and 2202 farm visits. Some key results are summarized below:

- More than half of the deliveries were to pig farms (59%). The rest were to poultry (26%), ruminant (14%), or farms with multiple types of animals (1%).
- The majority of deliveries were for bulk feed only (84%); the remaining deliveries were for bags only (6%), or for bulk and bagged feed (10%)
- The truck driver didn't enter the barn 93% of farm visits. The rest of the time, the driver entered the office (1%), the barn (5%), or an area with animals (2%)
- The farm lane was clean on 82% of farm visits. In some cases, though, the driver noticed mud and/or puddles (20%), manure (2%), or dead animals (1%)
- Dead stock management was adequate on 91% of farm visits, however the driver noted dead stock where he/she had to drive or walk on 3% of visits
- The feed bin area was clean 81% of the time, but there was spilled feed on 16% of farm visits
- Reusable rubber boots were worn on 21% of farm visits; the rest of the time drivers wore disposable (7%) or their own personal boots (72%).
- The boots cost between \$6.50 and \$16 per pair, and the cost of cleaning boots was \$1.75 per pair

BENEFITS TO THE SWINE INDUSTRY – This project has encouraged feed company personnel and swine producers to think about the issue of biosecurity in the feed industry. In particular, increasing awareness of biosecurity among the different players, and encouraging producers to be aware of what they can do to protect the industry as a whole. Overall, the participants appreciated our approach, and have expressed keen interest in understanding their place in this issue.

ACKNOWLEDGEMENTS – We are grateful for the participation of the feed company personnel and swine producers who took part in the focus groups and key- informant interviews, and in particular to the truck drivers for the pilot study. Thank you to Ontario Pork and the Ontario Agri Business Association for their assistance in recruiting participants, and to the Canadian Swine Health Board for their financial support.

A comparison of three animal welfare assessment programs on Canadian swine farms

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As animal welfare becomes a more important topic around the world, the assessment of animals on farm has become a more common practice. Various assessments have been developed around the world for different species of animals. Assessments all have the underlying goal of assessing the welfare of animals on farm; however, they may vary in their goals and in their overall composition. Standard measures used in animal welfare assessments include animal-based measures (ABM) obtained by observing animals (e.g. body condition score), resource-based measures (RBM) obtained by observing facilities (e.g. non-slip flooring in walkways), and management-based measures (MBM) obtained by interviewing farmers and checking records (e.g. written euthanasia plan). It is widely accepted that it is easy to train assessors to use RBMs and MBMs with good reliability, whereas ABMs are considered to be more direct measures of animal welfare but more difficult to achieve good agreement among assessors. However, few measures have been systematically tested. Our objectives were first to investigate inter-observer reliability (IOR) of different measures used in 3 current pig welfare assessment programs (the PQA Plus from the USA; the Canadian Animal Care Assessment (ACA); and the European Welfare Quality (WQ)), and secondly to determine the concordance of scores across the 3 assessments.

Data were collected on 5 grow-finish farms using a group of 10 trained assessors who conducted assessments on the same farms simultaneously. Individual dichotomous MBMs and RBMs (found primarily in the ACA and PQA) were analyzed quantitatively for inter-observer reliability using the kappa statistic. WQ was analyzed using Kendall's W.

Cohen's chance corrected kappa and Kendall's W values for the four observers present on all farms				
	Statistic	Including Pilot	Excluding Pilot	p-value
ACA	Kappa	0.86573	0.87465	0.05
PQA	Kappa	0.84833	0.84503	0.05
Welfare Quality	Kendall's W	0.754156	0.761624	<0.0001

These values show a very high level of agreement for data from 4 assessors who were the only observers present on all six farms (1 pilot farm, 5 assessment farms). The three assessments all examine similar welfare criteria (e.g. lack of hunger/thirst) but the ACA and PQA primarily use MBMs and RBMs, and WQ uses primarily ABMs. Therefore, overall results (ACA and PQA=Pass/Fail, WQ= Excellent, Enhanced, Acceptable or Not Classified) on 20 farrow-to-finish farms done by 2 observers were compared qualitatively to determine concordance among the 3 assessment programs in scoring individual farms. For 4 sample farms WQ scores categorized the welfare of the pigs on those farms as "Enhanced" whereas ACA and PQA resulted in "Failing" scores for those same farms. The results from both parts of the project indicate that MBMs are, in fact, highly reliable, but may not be concordant with ABMs.

Animal welfare assessments are increasingly becoming mandatory. As of last year, the ACA is mandatory in Canada for all swine producers. The results of this study can be used to identify what the best measures are to include in an animal welfare assessment program for swine to help improve the quality of the assessments being used today. The more efficient a welfare assessment is, the less disruptive it will be to the daily running of a farm when the assessment needs to be performed.

We would like to thank our sponsors (the Canadian Swine Research and Development Cluster and Ontario Pork), our collaborators in the various provinces (Manitoba Pork, Saskatchewan Pork and Le Centre de développement du porc du Québec), the additional observers in the various provinces, and all of the farmers who graciously allowed us access to their farms.

Dynamics of whole body nitrogen retention and blood urea nitrogen in young pigs challenged with 3bacterial lipopolysaccharide

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Background: Research in our laboratory has shown that feeding costs after weaning can be reduced without negatively affecting pig growth performance to market weight. This is achieved by reducing the use of complex animal proteins, such as blood plasma, fish meal, and whey, and increasing the use of soybean meal. However, before recommendations can be made to reduce diet complexity in the nursery, the impact of post-weaning nutrition on the ability to respond to an immune challenge should be explored. The objective of this study was to examine the effects of diet complexity on the dynamics of whole body nitrogen (N) retention, blood urea nitrogen (BUN), insulin-like growth factor-1 (IGF-1), and acute phase proteins (e.g. indicators of immune response) in young pigs following an endotoxin challenge.

Methodology: Eight pigs were weaned from Arkeil Swine Research Station and randomly assigned to either simple or complex diets (n = 4). Seven days post-weaning, catheters were surgically inserted into a jugular vein. Seven days post-surgery, pigs were moved to metabolism crates and fed restricted amounts of the experimental diets (450 g/d). Ten days post-surgery, bacterial lipopolysaccharide (LPS) was infused intravenously for 20 h ($5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and an N balance, reflective of lean tissue gain, was conducted. Feces, urine, and blood were collected daily until the end of the 8 day N balance period. N content of feces and urine was analyzed, and BUN, IGF-1, and albumin and haptoglobin were measured in serum.

Results: The LPS challenge transiently decreased whole body N retention, circulating IGF-1, and serum albumin, and increased BUN ($P < 0.01$). Pigs fed simple diets had higher BUN pre-challenge and post-challenge, and BUN was elevated post-challenge relative to pre-challenge levels. Diet complexity affected serum haptoglobin ($P < 0.01$) but did not affect whole body N retention, circulating IGF-1, and serum albumin following the LPS challenge ($P > 0.10$). The endotoxin infusion model did not elicit persistent metabolic changes in these pigs.

	Diet			P-value		
	H	L	SE	Diet	Time	Diet×Time
ADG (g/d)	287	319	14	0.171	-	-
N retention (g/d)	7.70	7.93	0.23	0.478	<0.001	0.800
BUN (mg/dL)	25.7	39.3	4.2	0.026	<0.001	0.983
Albumin (g/mL)	30.2	30.0	0.6	0.788	<0.001	0.707
Haptoglobin (g/mL)	1.11	0.65	0.12	0.008	0.472	0.999

Benefits to the swine industry: In this study, diet complexity did not affect the response of young pigs to an endotoxin challenge. It might therefore be possible to reduce diet complexity and feed costs in the nursery without adversely affecting pig health or growth performance.

Acknowledgements: Financial support was provided by Ontario Pork, the Ontario Ministry of Agriculture, Food and Rural Affairs, and Swine Innovation Porc.

The molecular association between health and growth performance of nursery pigs

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Background: Insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and growth hormone receptor (GHR) are important members of the IGF system and are critical for growth and development in animals. It has been observed that differential expression of IGF-1, IGFBP-3, and GHR is associated with certain infectious diseases and health ailments in animals. Recent findings suggest there is a complex interaction between these growth factors and the cytokine immune response. However, the molecular mechanisms behind this interaction are not well understood. It is proposed that elevated levels of inflammatory cytokines and acute-phase proteins (APPs), as a result of disease or stress, may compromise piglet development due to an unfavourable interaction with IGF-1, IGFBP-3, GHR, and other members of the IGF system.

Objective: To determine whether the hepatic gene expression of IGF-1, IGFBP-3, and GHR is significantly associated with the hepatic gene expression of CRP, SAA, Hp, IFN- α , IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, and IL-18 in nursery pigs from commercial farms.

Materials and Methods: RT-qPCR was completed on liver tissue from 74 piglets (5-weeks post-weaning) from 4 commercial farms in southern Ontario. Growth performance and disease status were also determined for each piglet. The data was used to build three multivariable logistic regression models in STATA 10.0 whereby IGF-1, IGFBP-3, and GHR were modelled as dependent variables, and cytokine expression, APP expression, health status, growth performance, and other piglet and farm characteristics were modelled as independent variables.

Results: Pigs with increased hepatic expression of IGF-1 were more likely to have increased ADG and increased IGFBP-3 expression ($P < 0.05$), but were significantly less likely to have PRSSV, increased TNF- α expression, and increased IL-18 expression ($P < 0.05$). Pigs with increased hepatic expression of IGFBP-3 were more likely to have an increased weight at weaning, increased IGF-1 expression, increased GHR expression, and increased IL-6 expression ($P < 0.05$). It was also determined that IGFBP-3 expression was associated with IGF-1 expression when Hp expression was low ($P < 0.05$), but this association was not significant when Hp expression was high ($P = 0.54$). Pigs with increased hepatic expression of GHR were more likely to have increased expression of IGFBP-3 and CRP ($P < 0.05$), but were less likely to have increased expression of IL-1 β and SAA ($P < 0.05$). Overall, there appears to be an inverse association between the hepatic expression of the IGF system (IGF-1, IGFBP-3, GHR) and certain cytokines and APPs (IL-1 β , IL-18, TNF- α , SAA, Hp).

Take Home Message: Poor swine health can result in elevated levels of inflammatory cytokines and APPs which may negatively impact growth and development in nursery piglets by suppressing the IGF system.

Acknowledgements

We thank Swine Innovation Porc for the financial support as well as Ontario Pork and the swine producers who participated in the study.

MICROBIOME OF THE TONSIL OF THE SOFT PALATE OF SWINE--POSSIBLE ROLES IN HEALTH AND DISEASE

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BACKGROUND: The last decade has seen a tremendous increase in the use of culture-independent techniques to characterize microbes present in humans and animals. Much of this work is concerned with the role that these microbial communities play in health and disease. For example, it has been shown that microbiome members can provide protection against infection through competitive exclusion or can contribute to disease by providing nutrients to pathogens. In pigs, the role of microbiomes in nutrition has been studied, but complex bacterial communities in the upper respiratory tract are only beginning to be investigated. In addition to harbouring a large number of poorly-characterized commensal organisms, the tonsil of the soft palate in swine is known to be the reservoir of many primary and opportunistic pathogens, but much remains to be learned about interactions at this site.

METHODOLOGY: Swabs and tonsillar samples were obtained from unfit animals in closeout groups and from healthy pigs at slaughter. Routine microbiological analysis was performed and both tissue and culture samples were characterized by Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis using the Phusion® Bacterial Profiling kit from Finnzymes. The distribution of T-RFLP fragments in healthy and unfit pigs was compared and tentative identifications were made by searching the Finnzymes, RDP, and a custom “pig-specific” database. Cluster analysis of the OTU data of healthy and unfit pigs was done to see if there was an association with 13 clinical signs or with the presence of PRRSV, PCV2, or *Mycoplasma hyopneumoniae* infection.

RESULTS: When the data were analyzed as anonymous OTUs, greater diversity was seen in the microbiomes of unfit vs. healthy animals. Comparison of the T-RFLP results with clinical data revealed significant associations with the presence of anemia, abscess, PRRS virus, and *Mycoplasma*. For example, a statistically significant association was seen between abscess and membership in clusters II and IX while membership in clusters III, VI, and X was positively associated with the presence of PRRSV. Putative identifications of the T-RFLP bands were done using several databases. At the phylum level, the microbiomes of unfit pigs were more likely than those of healthy animals to contain *Actinobacteria* and *Fusobacteria* and less likely to have *Firmicutes* and *Spirochaetes*. At the genus level, *Streptococcus* sp., *Flavobacteria* sp., *Clostridia* sp., *Lactobacillus* sp., *Bacillus* sp., and *Fusobacterium* sp. were present in more than 50% of both healthy and unfit animals although *Streptococcus* sp. was more frequently found in healthy than unfit animals (94.4 vs. 69.2%, respectively).

BENEFITS TO THE SWINE INDUSTRY: This work provides information about the bacterial communities present in diseased and healthy pigs and allows us to begin to identify organisms that might promote pig health or exacerbate disease.

ACKNOWLEDGEMENTS: This work was supported by the OMAFRA Animal Health Strategic Investment program and the Natural Sciences and Engineering Research Council.

GENETIC DIVERSITY OF HAEMOPHILUS PARASUIS

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BACKGROUND. Glässer's disease, caused by the bacterium *Haemophilus parasuis*, is a major source of loss in the swine industry. The majority of pigs carry *H. parasuis* in their nose, most frequently without any sign of disease. Our lack of understanding of the factors contributing to the development of Glässer's disease makes its control difficult. It is assumed that environmental, host, and pathogen factors interact to trigger the disease, but these factors are still unknown. The objectives of this study were to develop and apply a new efficient typing method for *H. parasuis*, and to assess the presence of associations between newly described virulence factors and the propensity of *H. parasuis* strains to cause disease.

METHODOLOGY. A *H. parasuis* collection consisting of 54 clinical isolates, 25 isolates from the nose of healthy pigs, and the reference strains of the 15 known serotypes was setup. A new multilocus variable number of tandem repeat analysis (MLVA) scheme was developed and used to type the 94 isolates from the collection. The isolates were also tested by PCR for the presence of 9 recently described putative virulence genes. The presence of association between the source of the isolates (i.e. sick versus healthy pigs) and specific MLVA types, clusters of types, or putative virulence factors was assessed.

RESULTS. Fifty-four MLVA types were identified among the 94 isolates tested (discrimination index of 97.4%). These types clustered into two major genetic groups. Serotype 5, 13, and 14 all belong to one of the groups and serotype 2 isolates to the other, while isolates of serotype 4 were evenly distributed between the two groups. A strong association was observed between seven out of the nine putative virulence genes investigated and one of the two genetic groups. However, no significant association was observed between the source of the isolates and the two major genetic groups or the presence of specific virulence genes. One single MLVA type lacking the majority of the putative virulence genes investigated contained 20% of all the clinical isolates examined. The results of this study also suggest that exchange of genetic material occurs between strains within each one of the two major genetic groups, but not frequently between the two groups. Overall, the results of this study demonstrate the diversity of the *H. parasuis* population present in Ontario and the ability of MLVA to type large numbers of *H. parasuis* isolates efficiently at an affordable cost. The results also show the lack of correlation between *H. parasuis* genetic groups or the presence of recently described putative virulence genes and the ability to cause disease in pigs. However, this study has identified a specific *H. parasuis* strains frequently involved in disease, which may be worth investigating in more details to identify what makes it different from the others.

BENEFITS TO THE SWINE INDUSTRY. An efficient and affordable typing method was developed in the course of this study, which will allow to trace *H. parasuis* strains and to provide a better understanding of the transmission and epidemiology of this pathogen at the animal, farm, and local level. Furthermore, this study has identified candidate strains for comparative genomics studies needed for the identification of new virulence genes potentially important for control strategies for Glässer's disease.

ACKNOWLEDGEMENT. This research project was funded by NSERC. We thank G. Chalmers, V. Nicholson, and G. Soltes for their technical assistance in this project.

Non-protein nitrogen increases nitrogen retention and feed efficiency in pigs fed a diet deficient in non-essential amino acid nitrogen

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INTRODUCTION

Estimates of ileal (upper gut) digestibility are widely used to estimate bioavailability of amino acids and crude protein (nitrogen [N] x 6.25) in pig feed ingredients. Nitrogenous compounds digested in the large intestine can be fermented by microflora with the production of ammonia. Absorbed ammonia is mainly used for urea synthesis in the liver and excreted primarily as urinary urea. In previous studies wherein non-protein N (NPN) was used to replace intact protein or essential amino acids in pig diets deficient in an essential amino acid, pig growth performance was not reduced. However, NPN may be used for the synthesis of non-essential amino acids by pigs and subsequently used for body protein gain. The objective of the present experiments was to determine the efficiency of using N absorbed in the large intestine (in the form of NPN) for N retention (body protein gain) and to demonstrate that feeding NPN can improve growth performance of pigs.

METHODOLOGY

Exp. 1 – N balance: A diet based on corn starch, casein and crystalline amino acids was formulated to meet requirements for all essential amino acids but to be deficient in non-essential amino acid-N. Nine barrows were infused either with saline or two different levels of urea-N (NPN; 1.5 and 3.0g/d urea-N) in the caecum via a simple T cannula.

Exp. 2 – Performance study: A negative control diet, based on corn starch, soybean meal and crystalline amino acids, deficient in non-essential amino acid-N was formulated (diet 1). This was supplemented with two levels of NPN (ammonium salts; diets 2 and 3). The positive control (diet 4) contained the same amount as available N as the high NPN diet, but N was derived from intact protein (casein). 12 gilts per treatment (2 per pen; 6 pens per treatment) were fed *ad-libitum* for three weeks.

RESULTS

Exp. 1: Urea-N infusion in the caecum improved N retention and body weight gain ($P < 0.05$). N excretion in feces and urine was not different between treatments ($P > 0.05$), implying that all N infused was absorbed and retained in the body. The efficiency of using infused urea-N for N retention did not differ between two urea infusion rates levels ($P > 0.05$; mean value of 96%).

Exp. 2: Overall body weight gain did not differ between treatments ($P > 0.05$), but feed efficiency was improved linearly with dietary supplementation of NPN ($P < 0.05$; 0.45, 0.47, 0.51 for diet 1, 2 and 3, respectively). Feed efficiency achieved with the highest NPN supplementation was not different from that of positive control diet ($P > 0.05$; 0.51 vs. 0.52).

IMPLICATIONS

Absorption of N in the large intestine should be considered in the formulation of low N diets. Diet crude protein or the non-essential amino acid N level should be considered when diets are supplemented with large amounts of crystalline amino acids.

ACKNOWLEDGEMENT

Financial support was provided by Evonik Industries, Ontario Pork, OMAF and Swine Innovation Porc.

The Use of Crude Glycerol as a Novel Feed Ingredient for Growing-Finishing Pigs

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Background:

Ontario farmers are becoming more involved in bio-diesel production; crude glycerol (also known as glycerine) is a co-product of bio-diesel production and can be used as an energy source for animal feeding. The production of one litre of biodiesel will yield approximately 80 grams of crude glycerol (CG) with the energy value of glycerol reported to be comparable to corn.

Objectives:

To determine the effects of feeding crude glycerol to growing-finishing pigs based on growth performance, carcass and meat quality.

Methodologies:

The experiment was conducted at Arkell Swine Research facility using 120 Yorkshire pigs (37.7 ± 2.5 kg initial BW), with 6 pigs per pen and 4 pens per dietary treatment. Diets contained either 0 (Con), 2.5(CG_{2.5}), 5(CG₅), 10(CG₁₀) or 15 (CG₁₅) % crude glycerol (90% DM basis), and were formulated to be similar in available energy and amino acid contents. The pigs were fed *ad libitum* with per pen feed usage and BW of individual pigs recorded weekly. Ultrasound measurements for backfat and lean depths were taken at 50 and 80 kg BW. After reaching the targeted 115 kg BW, pigs were transported to the University of Guelph Meat Science Laboratory and processed for an extensive carcass and meat quality evaluation.

Results:

- Days to market and ADG were similar ($P > 0.30$) for each CG level [ADG (kg/d): 1.08(Con), 1.11(CG_{2.5}), 1.07(CG₅), 1.07(CG₁₀), 1.10(CG₁₅); SE=0.03], while daily dry matter intake and dry matter (DM) to gain were also unaffected ($P > 0.30$) by feeding CG [DM/G: 2.44(Con), 2.40(CG_{2.5}), 2.44(CG₅), 2.51(CG₁₀), 2.53(CG₁₅); SE=0.07].
- At slaughter, hot carcass weight, dressing percentage, probe back fat and loin depth, ruler loin width and length, loin eye area, amount of lean in the loin as measured by dissection, and belly flex measurements were not affected ($P > 0.08$) by dietary treatment.
- Most meat quality measurements were not affected ($P > 0.08$) by feeding CG, while loin firmness was greater ($P < 0.02$) for CG₅ than CG₁₀ and CG₁₅ and was less for CG₁₅ vs. CG₁₀.

Results and benefits to swine industry:

Crude glycerol (glycerine) is not (yet) a CFIA approved feed ingredient for pigs in Canada. Therefore pork producers are not legally able to feed crude glycerol as a feed ingredient presently. Our work indicates that crude glycerol is a viable feeding option for growing-finishing pigs. The cost of crude glycerol will likely decrease in the future as glycerol supplies increase due to greater amounts of bio-diesel produced.

Acknowledgements: The authors would like to thank Ontario Pork, Growing Forward, Agricultural Adaption Council and OMAFRA/University of Guelph for their financial and in-kind contributions. Staff support at Ridgetown Campus – University of Guelph, the Arkell Swine Research Facility and the University of Guelph Meat Laboratory was also greatly appreciated.

Effect of prolonged anorexia on development of clinical signs associated with porcine periweaning failure-to-thrive syndrome (PFTS)

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Background: Porcine periweaning failure-to-thrive syndrome (PFTS) is a clinical condition, typically identified within 7 days of weaning, where affected piglets become anorexic, lethargic and then progress to debilitation requiring euthanasia (1). The estimated prevalence of PFTS is currently reported to be 4.3% with affected farms experiencing up to 20% mortality (2). The most commonly reported clinical signs associated with PFTS are anorexia (off-feed), oral chomping and progressive debilitation. However, research conducted to date has not been able to elucidate definitive risk factors, etiologic agent(s), effective treatments, or the pathogenesis, associated with the syndrome (3). It has also been hypothesized that the majority of lesions currently associated with PFTS are complicated by the histologic lesions attributed to the prolonged anorexia that PFTS-affected piglets experience. The objective of this project was to determine the role of prolonged anorexia on the development of the clinical signs and histologic findings currently associated with PFTS.

Methodology: This study was reviewed, approved, and monitored by the University of Guelph Animal Care Committee. Twelve piglets, all exactly 21 days of age, were randomly assigned to one of two treatment groups and matched by litter and sex. Piglets were housed individually. Group 1 (n=6) was fed a weaned pig diet ad libitum for 8 days. Group 2 (n=6) was fasted for up to 8 days. Ad libitum access to water was available to all piglets. Piglets were monitored twice daily and closely examined for evidence of hypothermia, weight loss/gain, general demeanor/posture, infectious disease, and behavioural changes. All piglets had blood samples taken 3 times during the study to measure changes in biochemical parameters. Environmental conditions of the room were monitored. At the completion of the trial all piglets were humanely euthanized and comprehensive post mortems were performed.

Results: All piglets in group 2 developed repetitive chomping/licking starting as early as 3 days fasted with all piglets demonstrating the behaviour by 5 days fasted. None of the piglets in group 1 developed repetitive chomping behaviour. The most predominant biochemical change noted between the groups was a higher level of beta-hydroxybutyrate (BHBA), a serum ketone, in the group 2 evident by 4 days fasted ($P < 0.001$, using multilevel linear regression). Two piglets in group 1 developed mild diarrhea by day 5. None of the piglets in group 2 exhibited any clinical disease. Body condition scoring was an insensitive method of determining which pigs were fasted, even after 7 days of fasting. This finding has been extrapolated to suggest that the historical cases of PFTS-affected piglets examined to date were likely anorexic for extended periods of time and were not identified in the early stages of the syndrome. This has potentially contributed to the inconclusive diagnostic work-ups and lack of response to treatments or interventions tried to date on farm. Results of the histologic findings are still pending.

Benefit to swine industry: This work will benefit the swine industry by enhancing our knowledge on how to identify PFTS-affected piglets in the early stages of the syndrome as well as how to interpret retrospective and prospective histologic findings. Improving our ability to identify piglets in the early stages of anorexia is imperative for our continued understanding of the risk factors associated with PFTS and its ultimate management or control. Additionally, the more timely identification of anorexic piglets in commercial nurseries will enable us to impose interventions and diagnostic tests sooner and improve animal welfare concerns associated with the prolonged anorexia whether or not associated with PFTS-affected pigs.

Acknowledgements: This study was funded by the Canadian Swine Health Board and the University of Guelph-OMAFRA Research Partnership.

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Does Type A *Clostridium perfringens* cause enteritis in neonatal pigs?

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Background

Attributing a pathogenic role to Type A *C. perfringens* in neonatal enteritis of piglets is problematic because of the marked colonization of the stomach and small intestine of piglets by *C. perfringens* within the first hours of life. Also, the gross and histopathological changes described in piglets diagnosed with Type A *C. perfringens* are variable. Therefore, the diagnosis of Type A *C. perfringens*-associated porcine neonatal diarrhea is traditionally based on the isolation of large numbers of *cpb2*-positive bacteria and the exclusion of other causes.

Objective

1) To compare the presence of Type A *C. perfringens* in diarrheic and healthy piglets; 2) to identify the frequency of neonatal diarrhea associated with Type A *C. perfringens* on Ontario swine farms between 2001 and 2010; 3) to determine the prevalence of Type A *C. perfringens* in normal pigs at different stages of production on Ontario farms; 4) to investigate the diagnostic methods for Type A *C. perfringens* in neonatal piglet enteritis.

Material and Methods

Thirty-six neonatal diarrheic piglets and 12 healthy piglets from 10 farms with a history of diarrheal illness were examined by histopathology, and tested for Type A *C. perfringens* as well as for *C. difficile* toxins, *Salmonella*, enterotoxigenic *E. coli* (ETEC), rotavirus, transmissible gastroenteritis (TGE) virus, and coccidia. In addition, data of 237 cases of 1-to 7-day-old piglets submitted for the diagnosis to Animal Health Laboratory (AHL) between 2001 and 2010 were analyzed. Also, 354 fecal samples collected on 48 Ontario swine farms were cultured for *C. perfringens*, and toxin genes were identified. Further, a questionnaire was administered to 22 veterinary practitioners to ask how they diagnose Type A *C. perfringens* infection and to 17 veterinary pathologists to collect information on how they confirmed a diagnosis for Type A *C. perfringens*.

Results

The numbers of Type A *C. perfringens* in the intestinal contents were even lower in diarrheic piglets compared with normal piglets ($P < 0.05$). The consensus *cpb2* gene was present in 93% of isolates in each group, but atypical *cpb2* was less common. *C. difficile* toxins and rotavirus were each detected in 33% of diarrheic piglets while the cause of diarrhea in 38% cases remained unknown. In the study of AHL data, a total of 155 cases were cultured for *C. perfringens*, and the organism was isolated in 86% cases. ETEC, Type A *C. perfringens*, rotavirus, and *C. difficile* accounted for 56% of the total cases while the etiology of 33% of cases was not identified. In addition, *C. perfringens* was isolated from 64% of 354 fecal samples (98% of suckling piglets, 34% of weanling pigs, 18% of grower-finisher pigs, 89% of gestating sows, 96% of lactating sows, and 75% of manure pit samples). Consensus and atypical *cpb2* genes were detected in 25 and 15% of isolates, respectively. Finally, the survey showed that pathologists generally diagnosed *C. perfringens* type A associated enteritis by various combinations of isolation of the organism, genotyping or detecting the toxins of the organism, and ruling out other pathogens through histopathology. The majority (95%) of veterinarians were moderately to very confident of their diagnosis; however, 41% of the pathologists were not confident of their diagnosis.

Conclusion

These findings indicate that the number of *cpb2*-positive *C. perfringens* in the intestinal contents is not a useful approach to diagnose Type A *C. perfringens* enteritis in piglets. The association between the presence of *cpb2* gene in *C. perfringens* and neonatal diarrhea in piglets remains unclear. It is possible that Type A *C. perfringens* enteritis is misdiagnosed in cases where no other pathogens are identified. In addition, the results of this study indicated that the current diagnostic methods used by practitioners and pathologists are not specific. Therefore, further research is required to determine whether Type A *C. perfringens* is a cause of neonatal enteritis in swine, and the basis on which the diagnosis can be made with confidence.

Acknowledgement

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Infrared thermography to evaluate lameness in pregnant sows

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Early detection of lameness in reproductive sows is important to reduce economic losses and improve animal welfare. Mild-to-moderate lameness is difficult to diagnose in sows. The objective of this study was to evaluate infrared thermography (IRT) as a method of detecting lameness in sows under farm conditions. A total of 297 sows were visually assessed using the following gait scoring system: 0) normal, 1) slightly lame, and 2) obviously lame. Obviously-lame sows were matched with score 0 and 1 sows. Sixty-five sows' rear limbs were examined by IRT. Sows were washed, dried and allowed to acclimatize to ambient environmental conditions. Images including the tarsus, upper and lower metatarsus and phalanges were obtained using a FLIR T300 camera. FLIR software was used to analyze temperatures. The associations between mean temperatures of the affected legs of different sows, temperatures of the affected and sound leg within a sow, and temperatures of the anatomical areas within the affected leg were analyzed using the Kruskal-Wallis test. IRT temperatures of the low metatarsi and the phalanges of the affected leg differed between score 0 and scores 1 and 2 ($P < 0.05$). The temperature between the phalanges and other anatomical areas, as well as the lower metatarsi and tarsi differed between score 0 and scores 1 and 2 ($P < 0.05$). The temperature difference between the affected and sound leg were not significant. The association of mean IRT temperatures of the affected leg with specific sow characteristics was also evaluated. Temperatures of sows with normal or straight limbs were lower than those with their hind limbs positioned too far forward ($P < 0.05$). Temperatures of the upper and lower metatarsi were lower in parity > 2 compared to parity 0 sows ($P < 0.05$). Obese sows had lower temperatures than normal/thin sows ($P < 0.5$). Phalanges and lower metatarsi were more affected by lameness. The application of IRT on pig farms may face unique sow anatomical and behavioural challenges. Additional studies are needed to confirm our results.

True Digestibilities and Endogenous Fecal Losses of Se and Zn in Growing Pigs

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BACKGROUND

Apparent fecal digestibility (AFD) values of dietary minerals may underestimate true fecal digestibility (TFD) values in pigs. The use of AFD in diet formulation can lead to overfeeding of minerals.

OBJECTIVES

The objectives of this study were to determine the TFD and the endogenous fecal losses (EFL) of Mg, Cu, Fe, Mn, Se and Zn in a corn/SBM-based diet in growing pigs by the difference method.

METHODOLOGY

Twelve Yorkshire barrows with initial BW of 23.9 ± 1.1 (mean \pm SD) were assigned to two dietary treatments in a completely randomized design. The two diets were formulated at 100% (HN diet) and 60% (LN diet), respectively, of the NRC (1998) requirements for Mg, Cu, Fe, Mn, Se and Zn. Feed and feces samples were collected during 5 d of ad libitum intake of the diets and analyzed for mineral contents by Inductively Coupled Plasma-Optical Emission Spectrometer. TFD was estimated from the difference in AFD between diets, and EFL was estimated from the difference between TFD and AFD.

RESULTS

DM digestibility of the HN diet (79%) was significantly lower than that of the LN diet (86%). However, the animals on the HN diet had superior ($P < 0.05$) average daily gain and feed efficiency compared with animals on the LN diet despite the fact that both groups of animals had similar ($P > 0.05$) average daily feed intake (ADFI). The AFD values of Se (73.9%) and Zn (9.5%) were significantly lower ($P < 0.05$) than their TFD values of 82.1% and 15%, respectively. Se and Zn EFL were 0.00004 mg and 0.01 mg/kg of DMI, respectively. The TFD values and EFL for Mg, Cu, Fe and Mn were not estimatable because of negative AFD values. It was concluded that the TFD values of minerals should be employed in diet formulation for the growing pig to avoid their mutual antagonisms during metabolism. Furthermore, the determination of TFD and EFL of minerals requires measurement of mineral intakes via the drinking water.

BENEFITS TO THE SWINE INDUSTRY

Formulation based on TFD of minerals would better support growth performance, reduce cost and avoid excess mineral intake leading to reduction of mineral export from the farm into the environment

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Generation of *Lactococcus lactis* capable of co-expressing epidermal growth factor and trefoil factor and its synergistic effects on epithelial cell migration

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Epidermal growth factor (EGF) and trefoil factor 3 (TFF3) peptides actively support the restitution and repair of mucosal epithelial barriers. Systemic and oral administration of the mitogenic peptide EGF to weaned pigs have been demonstrated to increase secretion and activity of digestive enzymes, demonstrating its role in differentiating gastrointestinal epithelial cells. The mitogenic peptide TFF3 is present in the small and large intestine and is up-regulated at sites of injury by stimulating cell migration to re-establish epithelial continuity. Combination treatment of EGF with TFF3 has been reported to synergistically enhance wound healing activity by increasing cell proliferation (EGF) and cell migration (TFF3). Recombinant expression of EGF and TFF3 using a probiotic microbe such as *L.lactis* is a potentially inexpensive alternative approach to chemical peptide synthesis for human or animal application. The aim of the present study is to generate *L.lactis* capable of co-expressing EGF and TFF3 and to examine the wound-healing effects of combination EGF and TFF3 treatment using an *in vitro* restitution model. Recombinant EGF and TFF3 was co-expressed and secreted by *L.lactis*. In bioreactor fermentations, EGF and TFF3 was produced at $26.8 \text{ ng}/\mu\text{l} \pm 3.0$ and $31.7 \text{ ng}/\mu\text{l} \pm 2.4 \text{ SEM}$, respectively. Epithelial HeLa cells treated with EGF and TFF in combination resulted in synergistically enhanced wound healing activity. **Benefits to the swine industry:** Resulting data establishes the potential feasibility of using *L.lactis* as a bioreactor to co-express and secrete biologically active EGF and TFF peptides for potential large-scale application to improve early-weaned piglet intestinal health. Future mechanistic studies will provide further understanding on the synergy role of these potent peptides on re-establishing intestinal mucosal integrity.

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Improving Soybean Meal Nutrient Value via Fermentation Using Newly Isolated Bacteria

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Background

Soybean meal continues to be a valuable source for protein in the adult animal feed but cannot be used to its full potential for piglets due to immature gastrointestinal tract which limits its ability to digest oligosaccharides, polysaccharides, and large proteins. Glycinin, one of the major proteins in the soybean meal stimulates local and systemic immune allergic responses and has negative effects on piglet performance. Past studies have showed encouraging results on the elimination of these antigenic and anti-nutritional factors, as well as an increase in essential amino acids, when soybean is fermented using bacteria. Overall piglet performance has also been found to improve when piglets are fed fermented soybean. The objectives of the current study are to improve the soybean meal fermentations by firstly isolating novel strains of bacteria that have high enzymatic activity via screening and test their efficacy to improve the fermentation of soybean meal.

Methodology

Bacteria screening and speciation: Bacteria were isolated from different fermented food sources and screened for protease, amylase, and cellulase activity. Bacteria with high enzymatic activity were selected and speciated using MALDI-TOFF.

Fermentation: The selected speciated bacteria were used to ferment soybean meal in an 85% moisture level. Microbial counts and pH measurements were taken at 0, 24, and 48 hours as a quality control. Fermented samples were lyophilized and homogenized for further analysis.

Protein profile analysis: Total soluble protein was measured using a DC Protein Assay. Proteins were then separated using SDS-PAGE and stained using Coomassie-Blue Staining.

Results:

Ten out of the hundred bacteria screened demonstrated protease, amylase, and/or cellulose activity. MALDI-TOFF results revealed some of these microbes to belong to the *Bacillus*, *Enterococcus*, *Lysinibacillus*, and *Staphylococcus* genera, with some of them being members of bacteria which have demonstrated probiotic property. SDS-PAGE results of the fermented soybean meal demonstrated a decrease in the allergenic proteins, while also increasing the amount of low molecular, more digestible peptides. Isolate 2 was observed to be the best at decreasing high molecular weight protein and thus the best isolated to efficiently improve the nutrient value of soybean meal. Further investigation of the effect of crude protein, amino acid profile, and oligosaccharide degradation is ongoing.

Benefits to swine industry: This research benefits the swine industry by effectively improving the soybean fermentation technique by making it an applicable, more cost effective, highly digestible, and a less allergenic, protein source to be used for piglets.

Acknowledgements: This work is supported by OMAFRA

Methicillin-resistant *Staphylococcus hyicus* isolated from pigs with exudative epidermitis carries the zinc resistance gene *czrC*

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Background

Staphylococcus hyicus is the causative agent of exudative epidermitis in pigs which is characterized by skin lesions and a greasy exudate. The disease is common and outbreaks of the disease can result in significant morbidity and mortality. Recently, methicillin-resistant *S. hyicus* (MRSH) has been identified which carries a Staphylococcal Cassette Chromosome *mec* (SCC*mec*); a mobile genetic element that harbors the methicillin-resistance gene *mecA*. It has also been discovered that the SCC*mec* of methicillin-resistant *Staphylococcus aureus* (MRSA) originating from pigs carries the zinc resistance gene *czrC*. It is predicted that MRSH may also carry *czrC* because it has been shown that the SCC*mec* is capable of horizontal gene transfer between different staphylococci species. Resistance to zinc is concerning because high levels of zinc oxide (≥ 2500 mg/kg) are commonly added to swine feed to control post-weaning diarrhea. This may create conditions that select for multidrug resistant staphylococci, resulting in swine diseases that are difficult and more costly to treat. The objective of this research is to determine whether MRSH also carries the *czrC* gene.

Methods

Staphylococcus hyicus isolates were collected in a previous study of 186 pigs that presented clinical signs of exudative epidermitis on 30 different farms in southern Ontario. Briefly, 124 isolates of *S. hyicus* were obtained and screened for phenotypic β -lactam-resistance by disk diffusion, and the *mecA* gene using PCR. In addition, SCC*mec* typing was completed on methicillin-resistant isolates. Overall, 28 MRSH isolates were identified from 28 pigs divided across 15 different farms. In the present investigation, the 28 isolates of MRSH were tested for the *czrC* gene using PCR amplification of genomic DNA.

Results

Fourteen (50%) pigs were colonized with MRSH that carried *czrC*, representing 8 (53%) of the 15 farms from which MRSH was isolated. Furthermore, 100% (14/14) of the MRSH isolates carrying the *czrC* gene were SCC*mec* type V. The finding that *czrC* is common amongst MRSH and distributed across multiple Ontario farms raises concerns because pigs are commonly exposed to high levels of in-feed zinc oxide. This exposure may cause selection of these multidrug resistant bacteria carrying *czrC*, and make treatment more difficult and costly for exudative epidermitis infections. In addition, of the pigs carrying *czrC* positive MRSH, 64% (9/14) were reported to be raised without exposure to antibiotics, and this association was statistically significant ($P < 0.05$, Fischer's Exact). This association was foreseeable as zinc oxide is a popular alternative to in-feed antibiotics, and zinc-resistance may explain the emergence of multidrug-resistant staphylococci on antibiotic-free farms.

Acknowledgements

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Dynamics of whole body nitrogen retention in gestating gilts at two feeding levels

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Background: Nitrogen (N) retention is a main determinant of amino acid and energy requirements of gestating sows. However, based on the most recent review, empirical data on the dynamics of whole body N retention in gestating sows is limited (NRC, 2012). Particularly for parity 1 and 2 sows, where maternal N gain is relatively large and affected by energy intake, more data is needed.

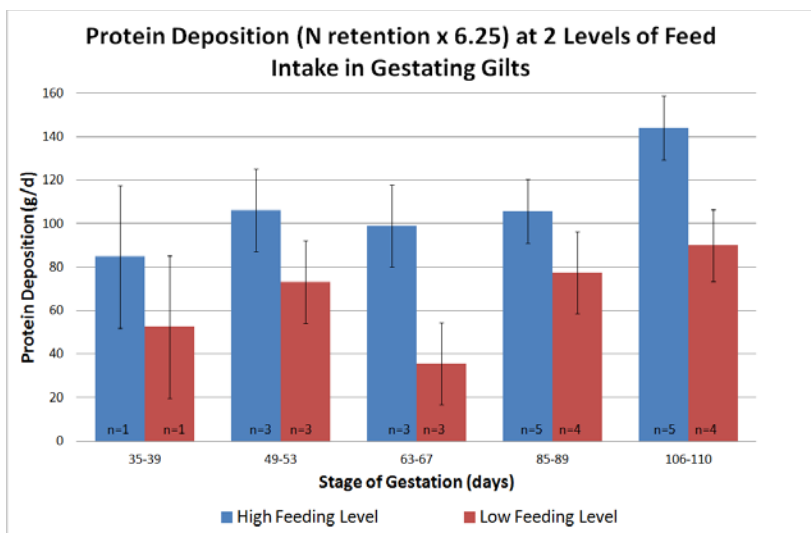
Methodology: To refine methodology for routine evaluation of dynamics of N-retention during gestation, 9 gestating purebred Yorkshire gilts at University of Guelph Swine Research Station were used during summer 2012. Each gilt was assigned to high or low feeding level (2.59 and 1.87 kg/day, respectively) when pregnancy was confirmed at 30 d of gestation. For both feeding levels a common corn and soybean meal based diet was used, containing 3.5Mcal/kg DE and 17.5% crude protein. Throughout gestation, there were 5 N-balance periods of 4.5 days in length and starting at 35, 49, 63, 85, and 106d of gestation. Urinary N was collected quantitatively via urinary catheters and fecal N was calculated from N intake and fecal digestibility; the latter was determined using a marker.

Results: Daily variation in urinary N excretion was observed within pig, period, and feeding level. The coefficient of variation (CV) within pigs and N-balance period ranged from 3 to 29% and 2 to 42% for the high and low energy intakes, respectively. Using at least 3 daily urinary N excretion values per pig and period, a targeted CV of less than 15% was obtained for average urinary N excretion per period. Feeding level had an effect ($P=0.0055$) on nitrogen retention. At both levels of feed intake, N retention increased towards the end of gestation; at 106-110 d it was higher ($P<0.01$) than at 63-67 d.

Benefits to Swine Industry: The differences between feeding levels and stages of gestation demonstrate the dynamic

changes in N retention during gestation in gilts. Determining the pattern of whole body N retention in gestating sows allows nutritionists to more closely estimate the changing nutrient requirements during gestation and formulate rations accordingly. Feed costs and excess nutrient excretion can be decreased, which poses a benefit to the producer and the environment.

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Mucosal bacteria associated with periods of reduced and compensatory growth in pigs

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Background: Previously we reported that growth was reduced in pigs fed low complexity starter diets and induced compensatory growth thereafter. The objective of the study was to 1) describe the ileal bacterial profile during periods of reduced and compensatory growth and 2) determine whether early nutritional insult permanently altered the profile of ileal mucosa bacterial populations.

Methodology: Alterations in ileal mucosal-associated bacteria in pigs fed high (H) or Low (L) complexity starter diets with or without in-feed antibiotics during periods of reduced (wk 2 post-weaning) and compensatory (wk 8 post-weaning) growth was evaluated using 16S ribosomal RNA (16S rRNA) gene pyrosequencing. Forty-eight pigs received one of four test diets at weaning (21 d of age) for 6 wk and common grower thereafter. Six pigs per treatment were killed at wk 2 and 8 for collection of ileal mucosal bacteria. Ileal sections were rinsed, washed vigorously to remove mucosal bacteria, and the wash was centrifuged to pellet the cells. Total DNA was extracted using a kit and amplified using eubacteria 16S rRNA primers, and further sequenced.

Results: Pyrosequencing resulted in a total of 311,245 and 349,524 sequences for an average of 12,780 and 14,427 sequences per sample at wk 2 and 8, respectively. Mucosal bacteria clustered more closely by wk post-weaning than starter diet treatment and there was no starter diet effect on bacterial diversity at wk 2 or 8. Firmicutes made up 91 and 96% of total reads at wk 2 and 8, respectively. The proportion of *Clostridium paraputrificum* increased ($P = 0.003$) from wk 2 to 8 in pigs fed L (0.8 vs $13 \pm 2\%$) but didn't change in pigs fed H (3 vs $6 \pm 2\%$). The proportion of *Clostridium leptum* decreased ($P = 0.02$) from wk 2 to 8 in pigs fed L (27 vs $1 \pm 6\%$) but didn't change in pigs fed H (11 vs $5 \pm 6\%$). Furthermore, the proportion of *Sarcina* genus tended to decline from wk 2 to 8 in pigs fed Low but tended to increase with wk post-weaning in pigs fed High ($P = 0.08$).

Significance to Industry: The increase in potentially beneficial bacteria and decrease in potentially pathogenic bacteria at wk 8 in pigs on L may, in part, explain the compensatory growth observed at wk 8 in these pigs. Early nutritional insult resulted in long-term alterations in the mucosa bacterial profile but the effect was species specific. This further stresses the importance of considering long-term changes in gut microflora when evaluating feeding strategies for young pigs.

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An investigation into the association between *C. perfringens* type A and diarrhea in neonatal piglets

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To investigate the possible role of *cpb2*-positive type A *Clostridium perfringens* in neonatal diarrheal illness in pigs, the jejunum and colon of matched normal and diarrheic piglets from 10 farms with a history of neonatal diarrhea were examined grossly and by histopathology, and tested for *C. perfringens*, for *C. perfringens* beta2 toxin, as well as for *Clostridium difficile* toxins, *Salmonella*, enterotoxigenic *Escherichia coli*, rotavirus, transmissible gastroenteritis (TGE) virus, and coccidia. *Clostridium perfringens* isolates were tested using a multiplex real time PCR to determine the presence of *cpa*, consensus and atypical *cpb2*, and other virulence-associated genes.

The numbers of *C. perfringens* in the intestinal contents were lower in diarrheic piglets (log₁₀ 5.4 CFU/g) compared to normal piglets (log₁₀ 6.5 CFU/g) ($P < 0.05$). The consensus *cpb2* was present in 93% of isolates in each group but atypical *cpb2* was less common (56% healthy, 32% diarrheic piglets isolates, respectively, $P < 0.05$). The presence of beta2 toxin in the intestinal contents of normal and diarrheic piglets did not differ significantly. *Clostridium difficile* toxins and rotavirus were each detected in 7 of 21 (33%) diarrheic piglets. Rotavirus, *C. difficile* toxins, *Salmonella*, or enterotoxigenic *E. coli* were concurrently recovered in different combinations in 4 diarrheic piglets. The cause of diarrhea in 8 of 21 (38%) piglets on 6 farms remained unknown.

This study demonstrated that the number of *cpb2*-positive *C. perfringens* Type A in the intestinal contents was not a useful approach for making a diagnosis of *C. perfringens* Type A enteritis in piglets. Further work is required to confirm whether *cpb2*-carrying *C. perfringens* Type A have a pathogenic role in enteric infection in neonatal swine.

The research was funded by the Ontario Ministry of Agriculture Food and Rural Affairs and the Animal Health Strategic Initiative.

Extended storage reduces n-3 PUFA content in trimmed pork loins and bellies

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Background: Rancidity and peroxidation of fats and fatty acids (FA) in pork products may reduce the content of n-3 polyunsaturated FA (PUFA) and may affect functional food attributes. Relatively few studies have evaluated the effect of storage on the FA content in pork products. The aim of this study was to determine the effect of extended storage on content of n-3 PUFA in ground trimmed loins (LO) and bellies (BE).

Methodology: A total of 56 pigs (4 barrows and 4 gilts per treatment, Landrace × Yorkshire × Duroc) were fed either flaxseed (FS) or fish oil (FO) containing diets during three different time periods (grower, G; finisher, F, or continuously, C; 25-65, 85-120 or 25-120 kg BW, respectively), resulting in six treatments. A 7th treatment consisted of a diet free of FS and FO that served as control (CON). The LO and BE were stored at – 20°C immediately after slaughter. Crude fat was extracted from LO and BE at 7 and 180 d of storage. Stability of fat and FAs was expressed as the percentage change in content within DM during storage.

Results and discussion: The cumulative n-3 PUFA intake was similar for the three FS and FO treatments ($P>0.10$; across G, F and C mean intake was 7 kg FS and 2 kg FO per pig). Within FS and FO treatments, crude fat content in BE did not change during storage ($P>0.10$; -1.20 and 1.47%, respectively), whereas crude fat content in LO tended to decrease ($P<0.10$) during storage for FS (6.99%) but not for FO (4.77%) treatments. Regardless of source and the time of feeding n-3 PUFA, total content of FAs decreased during storage ($P<0.001$; 61.0 and 29.4% for BE and LO, respectively). This coincided with reductions ($P<0.001$) in content of n-3 PUFA (64.9 and 38.5%) and n-6 PUFA (61.0 and 28.7%) in BE and LO, respectively.

Benefits to the industry: Extended storage reduces contents of FA in pork products, which may be attributed to rancidity-peroxidation reactions and would influence functional food attributes, and possibly consumer acceptance, of pork products.

Acknowledgement: Funding was provided by Ontario Pork, the Ontario Ministry of Agriculture and Food, and the Natural Sciences and Engineering Research Council of Canada (NSERC). Staff at Ridgetown College conducted the growth performance study.

A retrospective study of the etiological diagnosis of diarrhea in neonatal piglets in Ontario, Canada, between 2001 and 2010

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The passive laboratory surveillance data of the etiological diagnosis of neonatal piglet diarrhea between 2001 and 2010 from the Animal Health Laboratory, University of Guelph were analyzed to determine the relative importance and trends of different enteric pathogens associated with neonatal piglet diarrhea in Ontario.

A total of 237 cases that included live or dead 1-to 7-day-old piglets were submitted for the diagnosis of gastrointestinal illness between 2001 and 2010.

The combined frequencies for gastrointestinal illness cases involving *Escherichia coli*, *Clostridium perfringens* type A, rotavirus, and *Clostridium difficile* either as single pathogens or a complex of pathogens accounted for 56% of the total cases. A total of 33% of gastrointestinal illness cases did not have an etiological agent identified. The frequency of cases diagnosed with enterotoxigenic *E. coli* had a decreasing trend from 2007. Cases submitted in 2010 were more likely diagnosed with *C. perfringens* type A compared to cases submitted in 2002-2007 ($P < 0.05$). A significant trend for submission of samples in the winter was observed in cases diagnosed with *C. perfringens* type A, enterotoxigenic *E. coli*, rotavirus, and *Isospora suis* ($P < 0.05$). Enterotoxigenic *E. coli* was less likely diagnosed if *C. difficile*, *C. perfringens*, or rotavirus were detected ($P < 0.05$). Younger piglet cases were more likely to be diagnosed with *C. perfringens* type A ($P < 0.05$) and *C. difficile* ($P < 0.05$) than older piglets.

This study indicated that *E. coli*, *C. perfringens* type A, rotavirus, and *C. difficile* are enteric pathogens of concern for the Ontario swine farrowing operations, and that further research is required to understand the reasons for non-diagnosed cases.

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Effects of vitamin D supplementation at weaning on an Ontario swine farm

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Introduction: Piglets may be born with low serum concentrations of the vitamin D which may result in a predisposition to vitamin D deficiency (1). Indoor rearing of sows and their piglets, coupled with the fact that sow milk contains very low levels of vitamin D, makes it necessary to supply vitamin D via the diet (2). Creep feed is a common way to supplement vitamin D to the nursing piglet. However, the timing and amount of feed ingested is highly variable among individual piglets resulting in variable vitamin D levels in weaned pigs (3). It has been anecdotally suggested that low levels of serum vitamin D may predispose piglets to suboptimal post-weaning performance such as a decreased average daily gain (ADG) and increased risk of morbidity. This has resulted in the adoption of routine supplementation of piglets with oral vitamin D at weaning. However, there is limited data to support the practice of supplementing piglets with vitamin D to improve overall health and growth in the post-weaning period. The purpose of this study was to explore the relationship between serum vitamin D concentrations, ADG, and morbidity in weaned piglets during the first 28 days post-weaning on an Ontario commercial swine farm.

Methodology: One hundred and eighteen piglets, from one week's weaning on a farm experiencing high levels of post weaning morbidity, were individually identified and randomly assigned at weaning (ranging from 21-24 days of age) to 1 of 2 groups (Day 1 of trial). Group #1 (n=60) was the control group and they were administered 1 ml strawberry syrup orally. Group #2 (n=58) was the treatment group and they were administered 1 ml (1.042 g/ml) of commercial vitamin D syrup orally. Piglets were weighed, and blood samples were taken for vitamin D concentration analysis, on Day 1 and 28 of the trial. The piglets were observed daily for the 28 days after weaning and all piglet morbidity and treatments were recorded, and ADG calculated. Investigators were blinded to the treatment groups.

Results: Group #2 had a higher average vitamin D serum level when compared to Group #1 at Day 28 ($P<0.05$). However, there was no significant difference (multilevel linear regression) in ADG in the first 28 days after weaning between the two groups. There was also no significant difference in piglet morbidity (multilevel logistic regression) between the two groups. All analyses used statistical techniques to account for repeated measures, sex, parity, and weaning weight.

Benefit to swine industry: Supplementation of piglets with oral vitamin D at weaning resulted in higher average serum vitamin D concentrations when compared to non-supplemented piglets. However, the piglets with a higher serum level of vitamin D did not demonstrate any biological or performance advantage over the non-supplemented piglets in this study. The results do not support the management recommendation of routine supplementation of piglets with oral vitamin D at weaning in order to improve overall health or ADG in the first 28 days post weaning. Continued research involving randomized and controlled clinical trials, utilizing large numbers of piglets, is needed in order to demonstrate consistency of these findings.

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Modelling carcass dressing percentage in market weight pigs

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Background: When using models to optimize management strategies for growing-finishing pigs the prediction of carcass dressing percentage (**CDP**) is critical, especially when evaluating the use feed ingredients that contain high levels of non-starch polysaccharides (fiber; **NSP**).

Methodology: A total of 87 CDP observations (representing treatment means; range: 75.8-83.8%) from 17 different studies were used to develop CDP prediction equations. The following variables were considered: BW at slaughter (101-139 kg), pre-slaughter (7-day) feed intake (2.71-3.55 kg/d), pre-slaughter diet ME content (12.9-15.2 MJ/kg), pre-slaughter diet NSP content (8.96-29.4%), pre-slaughter NSP intake (**NSPi**; 0.25-0.91 kg/d), probe carcass loin depth (51.6-65.4 mm) and probe carcass backfat depth (**Fat**, 13.3-29.2 mm). Diet nutrient contents were recalculated according to NRC (2012) and the standard NRC (2012) ME intake curve was used to estimate pre-slaughter feed intake from reported feed intake. Gut fill was predicted based on previously conducted analyses using empty BW (**eBW**) and NSPi: Gut fill (kg) = $(0.379 + 0.486 \times \text{NSPi}) \times \text{eBW}^{0.463}$. To account for systematic differences between US and Canadian CDP, US CDP were increased by 5.8%. For the variables linear, quadratic and interactive effects were considered. The mixed procedure of SAS was used for parameter estimation, using study as a random effect.

Results and discussion: Based on Akaike's information criterion (AIC), Table 1 describes the best model fit ($R^2=0.9536$). The observed CDP varied between 75.80 and 83.80% (79.75 ± 1.74 , mean \pm SD), whereas the calculated CDP varied between 76.72 and 83.44 (79.75 ± 1.68 ; mean \pm SD). This study indicates that the best predictors for CDP were eBW, probe fat, and NSPi.

Table 1. Predicting algorithms to represent carcass dressing percentage (CDP)

Item	Coefficients	SE	P-value
Intercept	55.6461	24.1	0.024
eBW	0.6519	0.381	0.091
eBW ²	-0.00357	0.002	0.031
Fat	-1.4459	0.591	0.017
Fat x NSPi	0.3942	0.124	0.002
eBW x Fat	0.01151	0.005	0.020
eBW x NSPi	-0.09031	0.022	<0.001

Benefits to the industry: A relatively simple model can be used to estimate CDP in market weight pigs, and may be expanded to represent effects of gender (e.g. entire males and immune castrate males) and feeding ractopamine.

Acknowledgement: Funding was provided by Swine Innovation Pork

Effects of Prebiotics on Growth Performance, Health Status, Jejunal and Plasma Alkaline Phosphatase Kinetics and the Large Intestinal Fermentation in Weanling Pigs Fed Corn and Soybean Meal-Based Diets

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BACKGROUND

Ingestion of prebiotics such as inulin and resistant starch can have positive consequences, e.g., enhancing butyrate production and beneficial gut microflora without affecting digesta viscosity and thus digesta passage rate. Specific investigations into the use of soluble fibers in animal diets are needed to evaluate the impact on growth performance as well as the impact on changes to the intestinal environment and blood bio-markers for health.

OBJECTIVES

This study was conducted to determine effects of dietary supplementations of three prebiotics in replacing feed antibiotics on growth performance, blood urea concentration, fecal scores, jejunal and serum alkaline phosphatase (AP) kinetics and large intestinal fermentation in weanling pigs fed corn and soybean meal (SBM)-based diets.

METHODOLOGY

Experimental diets were formulated with corn (49%), SBM (28%) and fishmeal (9%) as the major bulky ingredients. A negative control (NC) was the basal diet, containing no antibiotics or supplemental prebiotics. A positive control (PC) was formulated by adding an antibiotic premix (lincomix 44 at 0.10%) in the basal diet at the expense of cornstarch. Three prebiotic diets were formulated to contain 0.75% of the three test prebiotics including retrograded resistant cornstarch, Fibersol-2 (a modified digestion-resistant maltodextrin) and inulin, respectively, at the expense of cornstarch. Yorkshire pigs, at 21 d of age, with an average initial body weight of 7.0 kg were allocated to floor pens with 6 pigs per pen, balanced for gender and litter, and fed one of the diets for 21 d according to a completely randomized block design [please see details in the Hayhoe M. M.Sc. Thesis (2013) at the University of Guelph].

RESULTS

There were no differences ($P>0.05$) in the growth performance endpoints, plasma urea concentration and the volatile fatty acid concentrations in the cecal and fecal samples among the treatment diets and between each of the treatment diets and the NC or the PC diet. Dietary supplementations of 0.75% fibersol-2 and inulin reduced ($P<0.05$) the V_{max} of the proximal jejunal AP compared with the NC diet. Furthermore, serum AP affinity K_m values were at least 10-fold higher than the K_m values of the jejunal AP.

BENEFITS TO SWINE INDUSTRY

Dietary supplementations of the three prebiotics at 0.75% had little effects on growth performance but might affect the gut and whole body health status via affecting intestinal AP detoxification kinetics in the weanling pig.

FUNDING SUPPORTS: Supported by Ontario Pork, Agricultural Adaptation Council and the OMAFRA-University of Guelph Partnership Research Program.

Whole body retention of 18:3n-3 and apparent conversion of 18:3n-3 to n-3 highly unsaturated fatty acids in pigs fed flaxseed or fish oil diets

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Background: Highly unsaturated fatty acids (**HUFA**) such as 20:5n-3 (**EPA**), 22:5n-3 (**DPA**) and 22:6n-3 (**DHA**) are important physiologically active compounds providing health benefits to humans. Pork can be enriched directly with n-3 HUFAs by feeding 18:3n-3 (**ALA**, natural precursor for n-3 HUFA) or n-3 HUFAs from flaxseed (**FS**) and fish oil (**FO**), respectively; that can be incorporated into inter- or intra-muscular fat pools. However, little is known about the retention efficiency (**RE**) of n-3 polyunsaturated fatty acid (**PUFA**) and apparent conversion (**AC**) of ALA to n-3 HUFA in pigs fed FS and FO containing diets during either the grower or finisher phases of growth.

Methodology: 56 pigs (4 barrows and 4 gilts per treatment, Landrace × Yorkshire × Duroc) were used in a serial slaughter study to determine the RE of 18:3n-3 and the AC of 18:3n-3 to HUFAs (18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3) in pigs fed either FS or FO containing diets over three different time periods (**G**, **F**, or **C**; 25-65, 85-120 or 25-120 kg BW, respectively), yielding six treatments. A 7th treatment, a diet free of FS and FO served as control (**CON**). Eight pigs were slaughtered at 25 kg BW to determine initial body composition. Empty BW (i.e., carcass plus viscera) was analyzed at 25 and 120 kg BW for fatty acid (**FA**) content.

Results and discussion: Within FS or FO treatments, cumulative n-3 PUFA intakes were similar across treatments ($P>0.10$; across G, F and C mean intake was 7 kg FS and 2 kg FO per pig). Growth performance and carcass characteristics were not influenced by dietary treatments ($P>0.10$). The content (mg/100 g empty BW) of the sum of n-3 PUFA was independent of FS treatments ($P>0.10$; 1112, 1231, 1057 for FSG, FSF, and FSC; respectively), higher ($P<0.001$) than FO treatments (620, 647, 635 for FOG, FOF, and FOC, respectively), and the lowest for CON (212). Expressed as a proportion of 18:3n-3 intake, RE of 18:3n-3 was similar for FSG, FSF, FSC, FOC, and CON ($P>0.10$; 60, 75, 68, 60, and 71%, respectively) and lower ($P<0.05$) than FOG and FOF (88 and 90%, respectively). Pigs fed FS had a lower n-6:n-3 PUFA ratio than FO ($P<0.05$; 2.6 vs. 4.4); both were lower than CON ($P<0.001$; 13). The AC of 18:3n-3 to sum of 20:5n-3 and 22:6n-3 was similar for FSG, FSF, FSC, and CON ($P>0.10$; 1.3, 1.1, 1.4, 1.8%, respectively), whereas no detectable AC of these FA were observed for FO treatments. The largest AC among n-3 HUFA was to 20:3n-3 which was lower ($P<0.01$) for FSG, FSL, FSC and FOC (8.4, 6.0, 6.4, and 7.8%, respectively) than FOG, FOF and CON (11.9, 12.2, and 13.6%).

Benefits to the industry: These results suggest that the content of n-3 PUFA from FS and FO reflects both the amount and type of FA deposited in the pig's body. Within FS and FO treatments, the n-3 PUFA content and AC to n-3 HUFA was independent of timing of feeding n-3 PUFA, which provides flexibility as to when n-3 PUFA can be fed to produce n-3 PUFA enriched pork.

Acknowledgement: Funding was provided by Ontario Pork, the Ontario Ministry of Agriculture and Food, and the Natural Sciences and Engineering Research Council of Canada (NSERC). Staff at Ridgetown College conducted the growth performance study.