

## 2016 University of Guelph Swine Research Day



The organizing committee is pleased to introduce the

#### **University of Guelph Swine Research Day.**

The Centralia Swine Research Update (CSRU) and the Mike Wilson Swine Research Day (MWSRD) have merged into this new partnership. The one-day program will highlight University of Guelph swine research and will carry on with the traditions of both the CSRU and MWSRD with high profile guest speakers, short updates on current swine research, written proceedings, and opportunities for networking.

In tribute to the CSRU there will be graduate student oral and poster competitions.

And, in tribute to the MWSRD there will be a

"Mike Wilson Keynote Presentation"

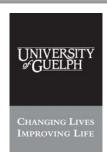
as well as a

"University of Guelph Faculty Feature Presentation".





Mike
Wilson
Swine
Research
Day



# 2016 University of Guelph Swine Research Day



The organizing committee wishes to acknowledge and sincerely thank the following sponsors

for their financial support of the program

**Animal Health Laboratory Bayer Healthcare** Bio Agri Mix LP **Boehringer Ingelheim Vetmedica BSC Animal Nutrition Conestoga Meat Packers Department of Animal Biosciences Department of Population Medicine Elanco Animal Health** Floradale Feed Mill Ltd **Gallant Custom Laboratories Grand Valley Fortifiers Kenpal Farm Products Inc Merck Animal Health Merial Animal Health Ontario Pork** Vetoquinol Wallenstein Feed & Supply Zoetis



# 2016 University of Guelph Swine Research Day

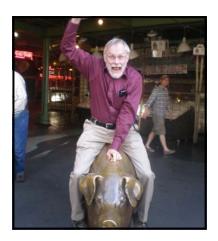


The organizing committee welcomes our guest speakers

#### **2016 Mike Wilson Keynote Presentation**

"Could animal production become a profession?"

Dr. David Fraser, University of British Columbia



David Fraser has maintained a strong interest in animals throughout his 44-year career of research and teaching in animal welfare and applied animal behaviour. In the 1970s he did some of the earliest research on animal welfare issues of pig production including studies of gestation stalls and early weaning. He then spent several years in wildlife research and established the role of highway de-icing salt in road accidents involving moose. In the 1980s and 90s he led a team of researchers dealing with the welfare of farm animals. Since 1997 he is Professor in the internationally respected Animal Welfare Program of the University of British Columbia in Vancouver. He has served as a scientific advisor to many organizations including the World Organisation for Animal Health (Paris), the Food and Agriculture Organization of the United Nations (Rome), and the Food Marketing Institute (Washington). In 2015 he was appointed a Member of the Order of Canada for his work as a pioneer in the application of science to animal welfare.

#### 2016 University of Guelph Faculty - Feature Presentation

"Solving the boar taint problem"

**Dr. Jim Squires, Department of Animal Biosciences** 



Dr. E. James (Jim) Squires completed his Ph.D. in Biochemistry in 1984 and then did postdoctoral work at the Laboratory of Pharmacology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina. In 1987, he came to the Dept. of Animal and Poultry Science, University of Guelph as an Assistant Professor and was promoted to Associate Professor in 1992 and to full professor in 1997. He became Chair of the Department of Animal Biosciences in April 2014. His research program is involved with the identification of candidate genes affecting important traits, especially those related to meat quality and metabolic disorders in livestock and the development of genetic markers for use in Marker Assisted Selection programs. He has supervised more than 35 graduate students and post-doctoral fellows and published more than 135 peer-reviewed manuscripts. In his presentation, Jim will discuss his research on controlling boar taint in entire male pigs and describe some of the key accomplishments he has made over the last 29 years. His work illustrates how research topics can come and go and come again as far as being urgent priorities, but meaningful long term advances in science can't be turned on and off. He will also discuss how new techniques like genomic technologies can shape a research program.

## The University of Guelph Swine Research Day Program

✓ follow the conversation on Twitter #UGSwineResearch

#### Wed May 4, 2016 8:30 AM – 4:30 PM Ontario Veterinary College, Lifetime Learning Centre Room 1714

8:30 am – light breakfast pastries and tea/coffee, pick up name tags and proceedings

#### **Morning Session**

**9:00** Welcome

**9:15** University of Guelph Faculty - Feature Presentation – Dr. James Squires, Department of Animal Biosciences – Solving the boar taint problem

10:00 Centralia Swine Research Update Graduate Student Poster Competition – 2 minute oral pitches

- POSTER #1 Touluwalope Ajayi Development of a prospective surveillance system for PEDV using multiple sources of data
- o POSTER #2 Emily Arndt Prevalence of various serotypes of *Streptococcus suis* in clinical cases and healthy-carrier pigs
- POSTER #3 Nicole Burello Kinetics of young porcine jejunal alkaline phosphatase in hydrolyzing endotoxin
- o POSTER #4 Emily Hill Fucose as a preservative of bioactive peptide
- O POSTER # 5 Danielle Hopkins An investigation into the relationship between influenza A virus (IAV) and *Streptococcus suis* infections in nursery pigs
- POSTER #6 Wilfredo Mansilla Dietary ammonia appearance in portal blood of pigs fed diets deficient in non-essential amino acid nitrogen is incomplete
- POSTER #7 Heather Reinhardt Growth performance and carcass quality in pigs fed a low or high complexity nursery diet

#### 10:20 Break and Poster Session

10:50 Centralia Swine Research Update Graduate Student Oral Presentation Competition – MSc

- 10:50 Holly Archer The identification of causative single nucleotide polymorphisms controlling boar taint
- o **11:05** Quincy Buis Development of precision gestation feeding program using electronic sow feeders and effects on gilt performance
- 11:20 Saranya Nair Study of Salmonella in naturally infected pigs from grower-finisher until slaughter

11:35 Centralia Swine Research Update Presentation - Doug Richards and Jaydee Smith

#### 11:45 Buffet Lunch and Poster Session

#### **Afternoon Session**

**1:00** The Mike Wilson Keynote Presentation – Dr. David Fraser, University of British Columbia – Could animal production become a profession?

1:45 Centralia Swine Research Update Graduate Student Oral Presentation Competition – PhD

- o **1:45** Russel Fraser Identification of germline variants in porcine innate immune genes using next-generation sequencing
- o **2:00** Golam Islam Encapsulated bacteriophage for the control of Salmonella in pigs at the primary production level
- o **2:15** Alison Lee Benefits of algae meal supplementation in nursery piglets

#### 2:30 Break

**3:00** University of Guelph Faculty - Research Presentations

- o **3:00** Dr. Renee Bergeron Animal Biosciences Recent swine welfare research and future directions
- o **3:15** Dr. Alfons Weersink Food, Agricultural and Resource Economics Prices and climate change
- o **3:30** Dr. Ray Lu Molecular and Cellular Biology Identification of genetic markers for stress-free pigs: lessons learned from lab mice
- o 3:45 Dr. Zvonimir Poljak Population Medicine Swine influenza research topics

**4:00** Centralia Swine Research Update Graduate Student Awards Presentation

**4:15** Closing remarks and adjourn

#### 5:30 pm - Evening Session

Social at the Bullring Pub

# Could animal production become a profession? David Fraser Animal Welfare Program University of British Columbia

Summary. Over the past 40 years, public concern over "intensive" animal production has triggered a growing series of regulations, standards and other programs that focus mostly on the environments in which animals are raised, with requirements for space allowance, air quality and other features. Although some of these requirements undoubtedly affect animal welfare, evidence now shows that very basic welfare outcomes – reflected for example in rates of disease, injury and mortality – vary widely from farm to farm even when the same kind of environment is used. These differences presumably reflect large differences in the quality of care that animals receive, likely because of differences in the skill, knowledge and dedication of producers and staff. The next step for improving animal welfare needs to focus on these human factors. Trusted professions, such as medicine and nursing, provide a model of work that focuses on the skill, knowledge and performance of people. There are steps that producers could take to make animal production more like a profession that is trusted and less like an industry that is regulated.

#### 1. Introduction

At the farm level, the term "animal welfare" makes us think of "animal care" or "animal husbandry" and it involves good nutrition, disease prevention, appropriate environments, low-stress handling, pain management and so on. These are the nuts and bolts of animal welfare, and they are the daily work of conscientious producers and veterinarians.

But over the past 30-40 years, animal welfare has also attracted the attention of governments, corporations and international agencies. For example:

- Starting in the 1970s, recommended national "Codes of Practice" for the care of animals on farms were developed, initially in the United Kingdom and later in other countries including Canada, Australia and New Zealand.
- In the 1980s, the European Union began to issue "directives" for the welfare of farm animals, initially for laying hens (1988) and soon after for pigs and calves (1991).
- In 1991 the American Meat Institute proposed a set of animal welfare standards and audit guidelines for slaughter plants. These and other requirements were then adopted by various fast-food restaurant chains and food retailers as requirements for their suppliers.
- In 1994, the RSPCA in Britain launched its "Freedom Food" labeling program (now re-branded as "RSPCA assured") so that customers could buy products from farms certified against specified production standards.
- In 2001, the World Organisation for Animal Health (OIE) began developing global standards for animal welfare, and today their flagship publication, the *Terrestrial Animal Health Code*, has over 100 pages of animal welfare standards.
- In 2006, the International Finance Corporation the investment arm of the World Bank issued a call for animal welfare to be part of the business plan of the companies in which they invest.
- And in 2008 the Food and Agriculture Organization of the United Nations (FAO) convened an international consultation on how to promote good animal welfare practices among livestock producers around the world.

As these examples show, animal welfare – while still rooted in on-farm animal care – has now become an issue of national, international and corporate policy. The question I want to

discuss in this presentation is how animal producers, veterinarians and other players should position themselves in a world where animal welfare has become an element of global business and policy.

#### 2. Industrialization

This will seem a strange place to start, but to understand the current debate about animal welfare I think we need to go back in time and think about the upheaval that occurred when the Industrial Revolution transformed agrarian society into industrial society.

One of the oldest ideas in our culture is that "agrarianism"— meaning rural life where people produce food and live harmony with the land—is the best kind of life for people. The idea was present in the classic civilizations of Greece and Rome, and has echoed down the centuries, as captured (for example) by the modern American writer Louis Bromfield who wrote: "The good farmer is one of the ultimate peaks of evolution... He is the one citizen without whom mankind and civilization cannot exist."

However, starting in the 1700s agrarian society began to be replaced through industrialization as factories became the main way of producing textiles and other goods in much of Europe. When that happened, people operating hand looms in their homes to supplement an agrarian livelihood could no longer compete with manufactured goods, and many were drawn or forced to move to crowded cities and work in dangerous factories. It was a profound change in society, and it set off a vigorous reaction by poets, artists, and reformers who praised traditional agrarian life and despised what the poet William Blake called the "dark Satanic mills" of industry. This reaction against industrialization influenced our culture in profound ways which continues to affect how we view life and industry today.

Of course, the critics could not turn the clock back to a pre-Industrial society, but there was a second concern about industrialization where reformers made real change. This focused on the welfare of the factory workers, many of them children and women, who were forced to work punishingly long hours in what we today would consider appalling conditions. In England, the response to these concerns was a series of laws called the "Factory Acts" which were designed to make the factory environments safer and healthier, and to limit the hours of work. As just a few examples from the England:

- An Act in 1802 required better ventilation and cleaning of textile factories.
- In 1819 an Act set a 12-hour work day for children up to 16.
- In 1847 dangerous machinery had to be fenced.
- In 1878 no children under 10 could work in factories.
- And in 1901 workers were to be protected from dust and fumes and to have 7 cubic metres of air space per person to allow adequate air quality.

In summary, then, industrialization triggered expressions of deep regret over the loss of agrarian life, and a reform movement that tried to protect the welfare of factory workers by regulating the factory environment and the time that people were exposed to it.

#### 3. The intensification of animal production

I believe that piece of social history actually set the stage for the response we have seen to the intensification of animal production.

First, just for sake of clarity, let's use the term "intensification" to mean four related things:

• consolidation of ownership so that animals are being produced on fewer, larger farms,

- some degree of confinement of animals in buildings or feedlots,
- some level of automation and cost-cutting, and
- genetic selection for efficient production under intensive conditions.

By those criteria the industrialized countries have undergone major intensification of animal production, but mostly in systems that use grain-based diets – especially pigs, poultry and eggs – whereas much less intensification has occurred in the forage-based systems for sheep and cattle.

To producers who use intensive systems, intensification basically means the "modernization" of their sector whereby production efficiency is increased through the use of specialization, automation, economies of scale and (now) electronic technology. People who see intensification in this way tend to support it, and some international development agencies actively promote intensification as a means of modernizing the food sector in poor countries and thus increasing food security and reducing rural poverty.

In the industrialized countries, however, much of the public sees intensification not as modernization but as industrialization. Farming is said to have become "factory farming"; modern agriculture is often referred to as "industrial agriculture". And this perception of intensification as industrialization has triggered a remarkable re-play of what happened during the Industrial Revolution.

Again, there were expressions of profound regret about the decline in agrarian life and the "family farm". Critics claimed, for example, "Replacing ... family farms are enormous factory farms owned by huge conglomerates." And, "In the past half century, animal agriculture in the U.S. has been taken over by corporations, turning family farms into factory farms." This concern about the loss of the family farm has played a large role in the debate about farm animal welfare. For example, in the California ballot initiative on confinement production methods in 2008, both sides claimed to be defending the family farm.

And as in the Industrial Revolution, there was also a second concern about how the new environments were affecting those inside them, but in this case the concern was focused not on the human workers – worthy as they may be of concern – but on the welfare of the animals. And the response followed the pattern of the old Factory Acts so closely that one British official called the early standards "a kind of Factories Act for animals".

As in the Factory Acts, many of the standards focused on the physical environment. For example:

- In 1988 a Swedish law prohibited the use of stalls for pregnant sows.
- In 2009 a directive of the European Union required 0.65 m<sup>2</sup> for pigs 85-110 kg and specified maximum slot widths for flooring for pigs of different sizes.
- In 2014 the new Canadian Code required at least 50 lux of light for at least 8 h per day and recommended that ammonia in the air be kept <25 ppm.

In addition, roughly paralleling restrictions on hours of work, we see restrictions on time in confinement. For example, since 2013 sows in the EU can be kept in stalls for only the first 4 weeks of pregnancy.

Thus we see a policy response to intensive animal production that followed very closely the response to concerns over the welfare of workers in factories. Essentially, intensive animal production was treated like a regulated industry with restrictions mostly on the physical environment and the time that the animals could be confined.

#### 4. Is this approach working?

But is this approach, with its emphasis on the physical environment and time in confinement, working to safeguard animal welfare?

Undoubtedly some of the standards will help. We know, for example, that high levels of ammonia lead to such basic welfare problems as inflammation of the eyes and respiratory system, and that pigs find high levels of ammonia so aversive that they avoid them if they can. Therefore, limiting ammonia levels seems likely to be of real benefit for animal welfare.

However, when we look at very basic welfare outcomes across a large number of farms all using the same type of physical environment, we see an important trend. Studies of piglet mortality provide an example. Piglet mortality rate is often recorded on farms because it is an important production statistic, but a high piglet mortality rate must also be seen as a major animal welfare issue because most piglets die from some combination of starvation, chilling and injuries. In Norway, where animal welfare standards require loose housing for sows at farrowing, a study of 39 farms found that the percentage of live-born piglets that died before weaning ranged from 5% on the best farm to 24% on the worst. A similar range of values was seen in a study of 30 farms in Canada (almost all with farrowing crates) which found that piglet mortality rate over 2 years ranged from 10% on the best farm to 32% on the worst. And a study of 83 farms in Europe, all following organic standards, reported piglet deaths ranging from 0 to 50% depending on the farm.

Wide variation from farm to farm is also seen in other outcomes relevant to animal welfare. A survey in Spain recorded bursitis and other variables on 30 farms, all using conventional growing-pig facilities with concrete floors. Bursitis was seen on only 7% of pigs on the best farm but 83% of the pigs on the worst, and severe bursitis ranged from 0 to 22% of pigs. And a study of sow mortality rate on 102 farms in France found that annual sow mortality ranged from 0% on the best farm to 20% on the worst, with little difference between farms using stalls and farms using tethers.

We also see the same pattern with other species. Just as examples, a study of 121 dairy farms, all using free-stall housing, found that the percentage of cows that were lame ranged from 5% on the best farm to 85% on the worst; and a study of broiler chickens, all housed in open barns with litter floors, showed lameness ranging from 0 to 90% of the birds depending on the company.

Why do we see this kind of extreme variation from farm to farm even when the same kind of physical environment is used? The answer is surely that animal welfare depends so much on the quality of the care that the animals receive. For instance, the risk of piglets dying can be reduced by many actions by the producer:

- providing a warm, draft-free environment for newborns,
- timely fostering of piglets from large litters,
- helping weak piglets to obtain colostrum,
- assisting sows in cases of prolonged parturition, and
- good hygiene to prevent mastitis because many piglet deaths occur in litters where mastitis reduces milk production.

Attending to all these factors represents a high standard of animal care, and it requires skill, knowledge and attentiveness by producers and staff.

Here we see an important difference between intensification and industrialization. When factory workers spend only a part of their days in factories, regulating the factory environment plus exposure time is a plausible way to deal with the welfare challenges that factories create. But when animals spend their entire lives under human control, good animal welfare relies less

on specific features of the physical environment (important as these are in some cases), and greatly on the skill, knowledge and attentiveness of the people. Thus if we want to improve animal welfare – along with food safety, environmental protection and other important goals – we need to foster and reward a high level of skill, knowledge and dedication in the people.

I believe this kind of evidence has brought us to a turning point in how we think about animal welfare. For 30 years, much of the focus has been on the animals' environment, and standards and regulations tended to emphasize features such as space, air quality and non-injurious flooring. These undoubtedly play a role in animal welfare. However, the very large differences in basic welfare outcomes from farm to farm suggest that human factors – including the skill, knowledge and attentiveness of producers and staff – play an overwhelmingly important role.

#### 5. Professional animal production

This brings me to the topic of "professional animal production", because animal production does not have to be either agrarian or industrial. As another option, we can look to the professions.

The professions are a kind of occupation that developed mostly during the last century and found its most sophisticated form in health care. In the 1840s, when Florence Nightingale announced that she wanted to become a nurse, by all reports her friends and family were horrified. Being a nurse at that time was a low-level service occupation and not a respectable role for a young lady. Nightingale went on to found the first school for nurses, and about 50 years later, progressive countries were starting to recognize the "Registered Nurse" as a profession. Doctors and surgeons have had an even more remarkable transformation. A century ago, owning a hundred beef cattle generated a greater income than being a mere doctor or surgeon, and probably reflected a higher status in society. But then the doctors and surgeons formed themselves into a profession and in that way generated a high level of respect and some obvious economic benefits.

What is a profession? They are not all the same, of course, but by most accounts professions include three elements:

- The main outcome is service to clients or the public.
- Participation requires competence, typically demonstrated to peers.
- And social license is maintained by adhering to the ethical expectations of society usually through some form of self-regulation within the profession.

This can be contrasted with a common industrial model where:

- The main outcome is products which the industries try to sell.
- Participation requires market success rather than competence demonstrated to peers.
- And social license often comes from obeying regulations because we generally regulate industries but trust professions to regulate themselves.

Given these three criteria, could animal producers re-shape their occupation to be more like a profession that is trusted, and less like an industry that is regulated?

First, are animal producers providing a service or just trying to sell products? Fifty years ago, the Green Revolution produced a surplus of cheap grain that triggered a rapid increase in animal production, and we saw the pork sector, the beef sector and the poultry sector competing for a greater share of the market. In that respect, animal production was clearly acting like an industry. However, we are entering a time when the demand for food will exceed current production because of world population growth, climate change and other factors, and simply

this increased need for food may well make food production look less like a group of industries competing to sell their products, and more like a profession providing an important service.

The second feature of the professions is demonstrated competence: people cannot join a profession unless they demonstrate their ability. Could this possibly be applied to animal producers? Again, fifty years ago this would have been inconceivable. The general belief was that "anyone can farm" and millions did. In Canada, for example, the 1941 census showed nearly a half million farms keeping pigs. Today fewer than 7,000 farms have pigs; in other words, the number of pig producers in Canada is about one tenth the number of doctors. Hence animal production has become a very specialized occupation where some practitioners have a high level of competence as we saw in the statistics coming from the best farms in the surveys described above.

Today, of course, many producers would consider it outrageous to require them to demonstrate their competence. However, we are seeing a growing trend for certification of farms according to standards for animal welfare, food safety and other issues. Some of these certification programs were created by specific retail companies, and were designed to promote confidence in specific product lines rather than the sector as a whole. However, the National Farm Animal Care Council has produced a framework for producer organizations to develop Animal Care Assessment Programs whereby producers themselves will manage programs to ensure compliance with the national Codes of Practice. As these assessment programs become developed and implemented, they could move animal production much closer to a professional model.

Third, could animal producers develop a self-regulatory system to show that they are adhering to the ethical expectations of society? A key step in this process is the development of standards that are seen as legitimate by society at large. Standards developed purely by an industry are likely to lack this kind of legitimacy, but the code development process of the National Farm Animal Care Council has three important features.

- It begins with a review of the relevant science which the code-drafting committee is required to consider.
- The code is then developed through an inclusive process that involves the humane movement, the veterinary profession and other players.
- And there is a period for public comment when people can express concerns which the drafting committee then considers.

If producers are sufficiently open and courageous in taking these broader inputs into account, then compliance with the Codes should reflect adhering to the ethical expectations of society.

Another step toward professionalism is seen in animal welfare enforcement. In a typical industrial model, public concerns are addressed by governments creating and enforcing regulations. In the case of animal welfare, however, we are now seeing some producer organizations making formal agreements with SPCAs and other regulators so that producers are actively involved in enforcement. If this process could go one step further – so that concerns about animal welfare routinely go to producer organizations and they call in enforcement in the tough cases – this would be much closer to the model of the medical profession dealing with their own disciplinary matters and the law becoming involved only as an exception.

In summary, in all three of these respects – service, competence, and social license – animal production is not yet functioning in the manner of a profession, but shifting toward a professional model looks more feasible than ever before. What would this achieve? First, over time I believe it would increase public trust in animal producers. Second, it would give animal

producers the leading role in the process. And third, I think it is the most promising way to improve animal welfare.

#### 6. Further reading

The first part of this talk is discussed in my book: *Understanding Animal Welfare: The Science in its Cultural Context*, published by Wiley-Blackwell in 2008, available from Amazon and other book-sellers. The second part is in a paper called "Could animal production become a profession?" published in 2014 in *Livestock Science*, which I would be happy to send on request at dfraser@mail.ubc.ca

## Solving the Boar Taint Problem E.J. Squires Department of Animal Biosciences, University of Guelph

#### What is boar taint and why is it important?

Boar taint is an off-odor and off-flavour in pork from uncastrated male pigs (intact males or boars), that makes it unacceptable to consumers. It is caused by the accumulation of two compounds, androstenone and skatole, in the fat. Androstenone is a steroid produced in the testis as the boar nears puberty, and it acts as a sex pheromone to regulate reproductive development in gilts and induce a mating stance in sows. Skatole is a bacterial breakdown product of the amino acid, tryptophan, in the gut. It is produced in equivalent amounts in the gut of both males and female pigs, but it is poorly metabolized and eliminated by males, so it accumulates in fat.

#### What are the current solutions for boar taint and why are they inadequate?

Piglet castration is a common practice carried out in the Canadian pork industry, mainly to avoid meat quality issues related to boar taint. However, raising intact males would present many advantages for pig producers including time and stress saved as a result of eliminating castration, better growth performance, better feed efficiency, higher carcass value, and improved health and welfare. In comparison to barrows (castrates), intact males have faster growth (+13%), leaner meat (+20%) and more efficient feed conversion (+14%) and consume less feed (-9.5%). Breeders and producers' production costs will be reduced so the producer/breeder of the intact male pig would get approximately \$10 more income than for a barrow; this amounts to an increase in profitability of pork production of 30-40%. The faster growth and greater feed efficiency will also have a positive environmental impact from reduced energy costs and less manure per unit of pork produced.

Many countries are gradually banning piglet castration, especially surgical castration without anesthesia and analgesia to improve animal welfare. For instance, the European Commission and representatives of European farmers, meat industry, retailers, scientists, veterinarians and animal welfare NGOs committed a plan to voluntarily end surgical castration of pigs in Europe by January 1st, 2018 (www.boars2018.com). Several countries have actually implemented a ban on boar castration ahead of the 2018 deadline (Norway, Switzerland), and a growing number of retailers now advertise that they only buy meat from non-castrated pigs. Controlling boar taint without surgical castration would, therefore, have dramatic benefits for production and consumer acceptance of pork products.

#### What at the potential novel approaches to control boar taint?

There are several promising alternatives to castration for dealing with boar taint. The amount of boar taint can be reduced by using a number of nutritional strategies and with good management practises. Boar taint can also be eliminated using an immunocastration vaccine. Genetic markers are also being developed to select for pigs that have reduced levels of boar taint.

**Nutritional manipulations to reduce taint.** Boar taint from skatole is affected by diet and environment (management) as well as the composition of the gut microflora. One of the main sources of tryptophan for skatole production comes from the turnover of cells lining the gut. Skatole

levels can be reduced by including sources of fermentable carbohydrates (chicory inulin, raw potato starch or high amylase barley) in the diet and by using various antibiotics to alter the gut microflora. Skatole can also be absorbed from the manure, so dirty pigs of any sex can have high skatole levels in fat.

Androstenone production is controlled by the sexual maturity of the boar, so diet does not have much of an effect on the production of androstenone. Androstenone levels could be decreased by slaughter of the pigs at lighter weights before androstenone synthesis increases at puberty, but this is not economically feasible. We have recently discovered that including binding agents in finishing diets can reduce the accumulation of androstenone in fat, by preventing its reabsorption from the gastrointestinal tract. We are now working to refine this technology and test it under commercial production conditions.

Immunocastration. Boar taint can be controlled by immunocastration using a vaccine (Improvest® from Zoetis) which stimulates the production of antibodies against gonadotropin releasing hormone (GnRH). GnRH is produced by the hypothalamus in the brain to drive the release of luteinizing hormone (LH) by the pituitary gland, which stimulates the development of the testis. The antibodies inactivate GnRH to shut down testicular development to the same extent as surgical castration and eliminate the production of androstenone and the sex steroids. Levels of skatole are also low in immunocastrated pigs, and this is most likely due to enhanced metabolic clearance by the liver after steroid production is suppressed, as occurs in surgically castrated pigs. Immunocastration also reduces the aggressive and sexual behaviour of boars. However, there has not been wide acceptance of this technology in North America or in the EU.

#### What is the ideal solution to control boar taint?

The use of genetic markers to produce lines of pigs that are free of boar taint but otherwise grow as normal boars is a long term solution to raising intact male pigs for pork production. Genetics can affect both the production and metabolism of boar taint compounds, and these effects can be found both within breeds and among different breeds. For example, levels of androstenone are much higher in Durocs than in the white pig breeds. There is also a wide variability in the amount of boar taint that individual pigs have within a breed. The heritability of both androstenone and skatole is moderate to high, but previous attempts to select for pigs with low boar taint have resulted in reproductive problems. The development of specific genetic markers for boar taint would minimize these negative effects on reproduction.

We have developed a panel of genetic markers to be used in breeding programs to select for low boar taint lines of pigs. These markers are based on SNPs (single nucleotide polymorphisms) in candidate genes that were selected based on functional studies we have carried out over the past 25+ years. In these studies, we characterized the metabolites and enzymes of the metabolic pathways involved in the synthesis and degradation of the boar taint compounds. We are now working with the Canadian Centre for Swine Improvement and provincial swine breeding organizations in Quebec, Ontario and Alberta to validate these markers in commercial swine breeds in Canada. This will produce a set of validated markers and guide the design for genetic evaluation programs to 'breed out' boar taint.

#### The identification of causative single nucleotide polymorphisms controlling boar taint

<u>H. Archer</u> BSc<sup>1</sup>; M. Jafarikia MSc, PhD<sup>2</sup>; B. Lillie DVM, PhD<sup>3</sup>; F. Schenkel MSc, PhD<sup>2</sup>; E. J. Squires MSc, PhD<sup>1</sup>. Department of Animal Biosciences, University of Guelph<sup>1</sup>, Center for Genetic Improvement of Livestock, University of Guelph<sup>2</sup>, Department of Pathobiology, Ontario Veterinary College<sup>3</sup>

**Introduction:** Boar taint, an unfavourable odour detected in the meat of intact male pigs, is caused by the accumulation of two compounds in adipose tissue; androstenone and skatole<sup>1</sup>. Despite mounting welfare concerns and an impending EU ban, surgical castration of all male piglets is still the most common control method of most production systems. The need for new methods in boar taint regulation is therefore a necessity. The aim of this study was to identify causative genetic mutations that can be used in selective breeding programs to effectively decrease boar taint production in pigs.

Methods: Genotyping was performed using a previously developed list of 120 single nucleotide polymorphisms (SNPs) on 3730 boars from three major swine breeds (Duroc, Landrace, Yorkshire). High Performance Liquid Chromatography (HPLC) and Enzyme Linked Immunosorbent Assay (ELISA) were performed on subcutaneous fat samples in order to determine skatole and androstenone concentrations, respectively. Association analysis was run to identify significant associations between genotype and the boar taint phenotype, using PROC GLM (SAS Version 9.4). Fixed effects consisted of SNP, sire, herd and season. Response variables, androstenone and skatole, were ln-transformed to account for skewed distribution of the data. Cytochrome P450 2E1 (CYP2E1) was selected for further study through gene expression analysis in a validation population of 63 boars. RNA was isolated from liver tissue and reverse transcription PCR was performed, follow by quantitative real time PCR (qPCR) targeting a portion of the coding region of the CYP2E1 gene. The association between CYP2E1 expression (ΔCT) and each SNP was analyzed. The effect of breed, androstenone and skatole concentrations on CYP2E1 expression were also tested.

**Results:** The mean ln(androstenone) level was 6.52 ug/g, while the mean ln(skatole) was 2.93 ug/g. Levels of subcutaneous fat androstenone and skatole were found to be significantly associated ( $\alpha < 0.0001$ ). Highly polymorphic regions for this phenotype were observed on SSC 6, 10 and 14. Genes with the highest frequencies of SNPs (EPHX2 & CYP2E1) corresponded to the latter of these chromosomes. In order to select for effective SNPs regulating boar taint, SNPs were eliminated based on the following criteria: MAF < 0.05,  $\alpha > 0.05$  and inverse effects for each compound. Breed effects

were proven influential for both androstenone and skatole levels alike ( $\alpha < 0.05$ ). qPCR analysis revealed significant individual variation in CYP2E1 gene expression. High linkage disequilibrium was observed among SNPs within the CYP2E1 promoter region. Breed had no effect on gene expression.

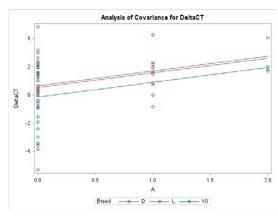


Figure 1: SNP Effect on CYP2E1 Expression

**Conclusions:** The candidate gene association study was successful in identifying 55 SNPs within 27 genes that are associated with levels of boar taint compounds. Additionally, 3 SNPs located in the promoter region of CYP2E1 were determined to have a functional role in the expression of CYP2E1, an enzyme that metabolizes skatole<sup>1</sup>.

**Industry Implications:** These promising results indicate the potential to use CYP2E1 promoter mutations in selective breeding programs in order to genetically select for boars with low boar taint phenotypes. In additional to controlling for boar taint, significant economic gains would be incurred due to the improved growth traits associated with raising intact males<sup>1</sup>.

**Acknowledgements:** Thank you to the Center for Genetic Improvement of Livestock for their collaborative efforts and data acquisition for this trial. Financial support was provided by Swine Innovation.

#### **Reference:**

1. Whittington F, *et al.* Relationships between skatole and androstenone accumulation, and cytochrome P4502E1 expression in Meishan X Large White pigs. Meat Science. 2004; 67: 569 – 576.

## Development of precision gestation feeding program using electronic sow feeders and effects on gilt performance.

R. Quincy Buis BSc; Douglas C. Wey BSc; Cornelis F.M. de Lange PhD Department of Animal Biosciences, University of Guelph

Introduction: Current commercial gestation sow feeding strategies do not consider the sow as an individual; they are generally based on using a single gestation diet for all sows regardless of parity or stage of gestation from breeding to farrowing. In this case, most sows are fed above their nutrient requirements for the majority of the gestation period, which in many cases can be costly for the producer and results in excessive nutrient losses into the environment. In addition, during late gestation and in young sows, when dietary nutrient requirements are highest, dietary nutrient levels may be below requirements. The latter may compromise long-term sow reproductive performance. Computer controlled electronic sow feeders (ESF) allow feeding of individual gestating sows housed in groups. With further development ESF technology has the potential to precision feed (PF) sows according to individual characteristics such as body weight (BW), parity, back fat (BF) and stage of gestation. Preliminary research was conducted to evaluate PF gestating gilts using the NRC (2012) nutrient requirement model.

The NRC (2012) model was **Methods:** adjusted to estimate daily energy requirements of gestating gilts, based on a constant daily lipid deposition target of 105 g/d, observed BW at breeding, assumed litter size of 12.5 and mean birth weight of 1.4 kg. 140 gilts were assigned at d2-9 post breeding to one of two dietary treatments, moved into group-housed ESF pens, and remained there until d101-107 of gestation. For half the gilts (PF), the feeding level and blend of two iso-caloric diets (NE 2518Kcal/kg; 0.80 vs. 0.20% available lysine for high and low protein, respectively; diets HP and LP) were adjusted daily for each animal to accurately meet estimated energy and lysine requirements. The remaining gilts (CON) received constant amounts of feed throughout gestation: 1.32 and 0.88 kg/d of HP and LP diets, respectively (mean available Lys 0.56%). Total feed allowance per sow (d3-105) was similar for both groups (PF vs. CON; 203.7 vs. 202.3 kg; *P*=0.74) while sows on PF used 6 kg less of the HP diet.

**Results:** Overall gestation BW and back fat gains did not differ between treatments (Table 1). As expected, gilts on PF tended to gain less in early gestation and more in late gestation. During the subsequent 21d lactation period, no treatment effects on performance were observed.

**Table 1.** Precision feeding and sow performance

Parameter	PF	CON	P		
Gestation BW gain, kg	63.9	60.2	0.17		
Gestation BF gain, mm	3.3	2.7	0.31		
Average daily BW gain, g/d					
early gestation (d5-32)	357	441	0.09		
mid gestation (d33-67)	700	709	0.86		
late (d68-103)	654	619	< 0.01		
Lactation performance					
Pigs born alive/litter	12.2	12.4	0.86		
Mean birth weight, kg	1.5	1.5	0.34		
Litter growth Rate, kg/d	2.5	2.6	0.35		
Sow feed intake, kg/d	5.1	4.7	0.14		
Sow BW gain, g/d	-789	-929	0.25		

**Conclusions:** In this study, PF gilts did not affect overall gestation BW and back fat gain or lactation performance. However patterns of BW gain for PF gilts more closely reflected the gains of fetus and reproductive tissues. A cost difference was not expected in this study as both groups received the same amount of protein and feed.

**Industry Implications:** ESF technology, that involves blend feeding of two basal diets, can be used to meet the unique energy and lysine requirements of individual sows in a group housing system. Long-term effects of this precision feeding technology remain to be explored.

**Acknowledgments:** Financial support from: OMFRA, Farms.com, Canarm Ag, sow choice systems. Technical support from: Arkell Barn staff, Lab mates and Technicians.

#### **Reference:**

1. NRC. 2012. Nutrient requirements of swine. 11th edition. National Academic Press, Washington, DC.

#### Study of Salmonella in naturally infected pigs from grower-finisher until slaughter

Saranya Nair BSc; Vahab Farzan DVM, MSc, PhD; Terri L O'Sullivan DVM, PhD; Robert M Friendship DVM, MSc, Dip ABVP

Department of Population Medicine, University of Guelph

**Introduction:** The presence of multi-drug resistant Salmonella in swine is a public health concern. Understanding the patterns of Salmonella shedding from the grower stage until slaughter is important when creating Salmonella prevention and control strategies. The objectives of this study was to determine how long pigs shed Salmonella if they enter a grower barn already naturally-infected and whether Salmonella can be detected from such pigs at slaughter.

**Methods:** Nine-week-old pigs (n=45) were purchased from a farm with a history of Salmonella infection. The pigs were housed at the Ponsonby Animal Research Facility, at the University of Guelph. Individual fecal samples were collected weekly for ten weeks starting when pigs entered the barn (week 0). Two to three weeks following the end of the trial, pigs were slaughtered (~86.8kg; average carcass weight) at the University of Guelph abattoir. Tissue samples (ileocecal lymph node, neck lymph node, spleen, liver, tonsils) and cecal content samples were collected from each pig. Fecal and tissue samples were cultured for Salmonella using Tetrathionate broth (selective enrichment) and XLT4 (agar plate). Agglutination test was used to confirm Salmonella isolates.

A multilevel mixed-effects logistic regression model was used to analyze the prevalence of Salmonella shedding. A Kaplan-Meier survival function and a Cox proportional hazard model were used to present and evaluate Salmonella shedding over the time.

Results: No clinical signs of salmonellosis were observed. Over the 10 weeks of sample collection all pigs tested positive for Salmonella shedding at least once and 89% of pigs more than once, with one pig testing positive 8 times. Out of the 45 pigs, 41 pigs were positive 4 times or less and 4 pigs tested positive 5 times or more. The highest prevalence of Salmonella shedding was at 10 weeks of age (week 1; 80%) and at 12 weeks of age (week 3; 91%). As pigs aged from 10 to 17 weeks of age, there was a significant decrease in Salmonella shedding (P<0.001) (Figure 1).

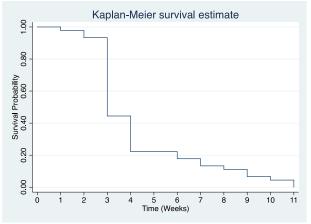


Fig 1: Kaplan-Meier survival estimate illustrates the time to event of when a pig stopped shedding Salmonella over 10 weeks

At slaughter, Salmonella was isolated from 7 pigs (16.3%). Salmonella was cultured from one or more of the following tissues: ileocecal lymph node, neck lymph node, spleen, liver, or tonsil, and isolated three times from cecal contents. Of 7 pigs harboring Salmonella at slaughter, 5 (71%) of those had not tested positive on weekly fecal checks for at least 7 weeks or longer.

Conclusion: This study found that asymptomatic carriers could continue Salmonella shedding for up to 8 weeks in the grower phase. The absence of Salmonella detection in fecal samples in pigs in the late finisher stage is no indication that Salmonella will not be found in tissues at the time of slaughter, posing a food safety concern.

**Industry Implications:** Because of prolonged shedding and the presence of unapparent carriers, it will be useful to implement intervention strategies such as vaccination at weaning to control Salmonella shedding during grower-finisher stage.

Acknowledgments: OMAFRA-FSRP, OMAFRA-UofG Research Partnership, Swine Innovation Porc and Huvepharma.

## Identification of germline variants in porcine innate immune genes using next-generation sequencing

Russell S. Fraser DVM, MSc; Hein N. Snyman BVSc, DVSc, Dip ACVP<sup>1</sup>; Ann Meyer PhD<sup>2</sup>; Jutta D. Hammermueller PhD; Brandon N. Lillie DVM, PhD, Dip ACVP
Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada
Current address: Animal Health Centre - BC Ministry of Agriculture, Abbotsford, BC, Canada<sup>1</sup>;
Ontario Institute for Cancer Research, Toronto, Ontario, Canada<sup>2</sup>

**Introduction:** Infectious diseases are a significant source of economic loss and welfare concern to the swine industry. Part of the multifactorial approach to diminishing the impact of infectious diseases is understanding the role of the porcine innate immune system. In previous studies of innate immune genes, we have shown that there are some genetic mutations that are more common in diseased versus healthy pigs, and that in healthy pigs, the levels of expression of innate immune genes differs significantly (1, 2).

The promoter region of a small subset of the differentially expressed genes was examined, and several contained single nucleotide polymorphisms (SNPs) associated with the presence or absence of disease (2). However, only a small segment of the promoter region in a small subset of genes could be investigated using traditional Sanger sequencing methods. In this study, we performed next-generation, targeted re-sequencing of 112 porcine innate immune genes and their regulatory regions to identify genetic mutations associated with varying expression levels.

Methods: DNA was obtained from the liver of 87 healthy, market weight pigs being processed at a large Ontario abattoir (2). The coding regions, introns, and up to 50 kb of upstream and 3 kb of downstream sequence of 112 innate immune genes were targeted for re-sequencing. Each of the 87 DNA samples was tagged with a unique 8-bp index, allowing for individual identification in downstream analysis. Target sequence enrichment was performed using custom probes from Roche Nimblegen and the library was sequenced over 2 lanes of an Illumina HiSeq (The Center for Applied Genomics, the Hospital for Sick Children, Toronto, Canada).

**Results:** The targeted 6.4 Mb of DNA was sequenced to an average depth of 14x per pig. After applying quality control filters designed to be inclusive, rather than restrictive, 26 965 SNPs were

identified, 847 of which had not previously been reported in a public SNP repository (dbSNP build 145). The distribution of the SNPs is presented in Table 1.

Table 1: Distribution of SNPs in 112 innate immune genes

Region	Total (novel)
Upstream (up to 50 kb)	11 405 (315)
Coding	558 (13)
Missense	281 (10)
Synonymous	252 (3)
Nonsense	2 (0)
Intron	11 498 (274)
Downstream (up to 3 kb)	3495 (245)

**Conclusions:** Using modern, high-throughput sequencing, this study has identified 26 965 SNPs spanning 112 porcine innate immune genes, 847 of which are novel. The relationship between the variation in expression of select porcine innate immune genes and these SNPs will be investigated.

Industry Implications: Any novel genetic mutations identified that are associated with decreased resistance to infectious disease could potentially be utilized in future breeding strategies. This could lead to reductions in the impact of infectious diseases on production parameters, welfare, and antibiotic usage.

**Acknowledgements:** Funding was provided by Ontario Pork, OMAFRA, an NSERC CRD, and the Canadian Centre for Swine Improvement, Inc., as well as graduate student funding from OVC.

- 1. Keirstead *et al.* Vet Immunol Immunpathol. 2011;142:1-13.
- 2. Snyman, HN. *DVSc Thesis*. 2013. University of Guelph.

Encapsulated bacteriophage for the control of *Salmonella* in pigs at the primary production level Golam S Islam BSc, MSc<sup>1,2</sup>; Qi Wang, MSc, PhD<sup>2</sup>; Joshua Gong MSc, PhD<sup>2</sup>; Robert M Friendship DVM, MSc, Dip ABVP<sup>3</sup>; Keith Warriner BSc, PhD<sup>1</sup>.

Department of Food Science, University of Guelph<sup>1</sup>; Guelph Food Research Center, Agriculture and Agri-Food Canada<sup>2</sup>, Department of Population Medicine, University of Guelph<sup>3</sup>.

**Introduction:** Salmonella is one of the leading causes of foodborne illness and is commonly encountered in pork. Concern on the emergence of multi-drug resistant strains such as S. Typhimurium DT104 is increasing. As a consequence there is a need for anti-Salmonella agents that can be used instead of, or in combination with, antibiotics(1,3). Bacteriophages have been long considered an effective treatment for Salmonella although phages can be inactivated by the stomach acid that ultimately limits efficacy(2). However, by encapsulating phages should in principle, be protected from stomach acid then released in the lower intestine where Salmonella resides(2). The objective of this study was to encapsulate bacteriophages specific to Salmonella Typhimurium DT104, test protection in simulated GI condition invitro, and evaluate release performance in pigs.

**Methods:** Four lytic bacteriophages specific to Salmonella Typhimurium DT104 were separately encapsulated by spray drying and mixed together to formulate encapsulated phage cocktail. A total of 18 newly weaned pigs were randomly selected and distributed among 3 treatment groups at a male to female ratio of 1:1. Two groups of 6 pigs were orally gavaged with ~10log pfu/g encapsulated phage cocktail and free phage cocktail respectively starting from day 1 till day 11, while the third group received placebo. Fecal samples were collected on day 0, day 2 and day 6 at 5h, 7h and 9h post gavage. Pigs were euthanized on day 12 and digesta contents were collected. Microbiological analysis was carried out to quantitate viable and released phages.

**Results:** *In-vitro* study in simulated gastric fluid (SGF) pH 2.0 for 2h inactivated free phages but over 90% infectivity was retained when encapsulated. In vivo test showed that from day 2 phage shedding was observed among 33%, 67% and 83% pigs administered with free phages at 5h, 7h and 9h post gavage, respectively. Alternately phage shedding was below detection limit (25pfu/g) among pigs in encapsulated

phage group but was detected among 100% pigs upon enrichment. On day 6 100% pigs shed phages at 9h post gavage while free phage shedding decreased from 83 % (7h) to 67% (9h) pigs. However, free phage titre was significantly higher (P<0.05) than the encapsulated phages on day 6. Phage titre recorded to be the highest ( $4.27\pm2.34$  pfu/g) at 7h and lowest ( $3.66\pm2.97$  pfu/g) at 9h among free phage group while released phage titre among encapsulated phage group was found to be the highest ( $4.25\pm0.47$  pfu/g) at 9h and lowest ( $4.91\pm0.82$  pfu/g) at 5h. At day 12, encapsulated phages isolated from jejunum region were significantly higher (P<0.05) than free phages.

Conclusions: Encapsulation protects bacteriophages against stomach acidity and delivers them into the small intestine. Temperature (<90°C) was found to be the key factor for phage survival during spray drying while the enteric polymer in the formulation helped protection to phage in acidic condition. Phage excretion in feces at high titre was evident among the free phage fed pigs due to segmented movement as early as 5h post gavage while encapsulated phage persisted in the GI tract followed by shedding at an incremental rate at 9h. Digesta contents of the pigs showed temporal distribution with a significantly higher encapsulated phage presence at the upper intestine.

**Industry Implications:** Large scale production of encapsulated phages can be achieved through a spray drying technique facilitating feeding the phage along with the feed in commercial farm settings as an antibiotic alternative to reduce Salmonella carriage among pigs.

**Acknowledgements:** Agriculture and Agri-Food Canada. OMAFRA University of Guelph Partnership.

- 1. Zhang J, *et al.* (2015). J Ani. Sci Biotechn. DOI 10.1186/s40104-015-0039-7
- 2. Yongsheng MA, *et al.* Applied and envt microbiology. 2008;74(15):4799–4805.
- 3. Toro H, et al. Avian Diseases. 2005;49:118–124.

#### Benefits of algae meal supplementation in nursery piglets

Alison V. Lee BSc; Lan You BSc; Se-Young Oh PhD; Rebecca E. Fisher-Heffernan PhD; Julia Zhu; Douglas Wey; Cornelius F.M. de Lange PhD; Niel A. Karrow PhD.

Department of Animal Biosciences, University of Guelph.

**Introduction:** Omega-3 polyunsaturated fatty acids (n-3 PUFA) are well-known for their anti-inflammatory properties. These n-3 PUFAs are essential nutrients, as longer chain fatty acids such as docosahexanoic acid (DHA) cannot be readily synthesized in mammals. Until recently, fish have been considered a major source of these long chain fatty acids. This has put a great strain on the fishing industry. Algae meal (AM) grown under strictly controlled conditions has therefore been suggested as alternative source of n-3 PUFAs. AM is equally rich in DHA and may provide a more sustainable source of omega-3 enrichment for growing animal diets. The aim of this study was to compare a control diet to diets supplemented with AM or fish oil (FO) in nursery pigs and evaluate the effects of n-3 supplementation on growth and stress response to a simulated bacterial infection.

Methods: Seventy-two cross-bred piglets were selected, weaned and placed on a low quality protein diet supplemented with either 1.25% FO or 3.12% AM or 5% Corn oil as a control for three weeks followed by a common diet for three weeks. Diets were matched for total n-3 content. Performance was measured over a 42-day period during the study. Eight pigs per dietary treatment were subjected to an *i.m.* LPS endotoxin stress challenge (50 μg/kg body weight) on days 21-23, with repeated blood sampling from jugular catheters to measure PUFA levels and cortisol concentrations throughout

the LPS challenge. Cortisol concentrations were measured using a commercial cortisol enzyme immunoassay.

**Results:** Different levels of PUFA enrichment in blood were detected after 21 days on feed. There were no significant differences found in piglet performance as measured by average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency between any of the dietary treatments. AM supplementation reduced peak cortisol levels following LPS challenge compared to both FO and control diets.

Conclusions: From this experiment we can conclude that AM supplementation in nursery pigs is comparable to FO in terms of growth performance and comparatively reduces peak cortisol in response to stressors.

**Industry Implications:** Results from this study suggest that AM may increase piglet robustness and allow for cheaper, lower quality nursery diets without compromising piglet health. AM may also be a more sustainable and environmentally friendly supplement than FO and as further research is conducted in this area, AM may become a more economical choice.

**Acknowledgements:** Funding provided by NSERC, OMAFRA, and Alltech Inc. AM was provided by Alltech Inc. Assistance with animal trials was provided by the Arkell Swine Research Station staff.

#### Development of a prospective surveillance system for PEDV using multiple sources of data

<u>Toluwalope Ajayi</u> BSc<sup>1</sup>; Zvonimir Poljak DVM, MSc, PhD<sup>1</sup>; Rozita A. Dara MSc, PhD<sup>2</sup> Department of Population Medicine, University of Guelph<sup>1</sup>, School of Computer Science, University of Guelph<sup>2</sup>

**Introduction:** The detection rate of novel species and variants of viruses in domestic pigs at a global level increased from 1.8 per year before 2000 to 4.5 new agents per year after 2000, while the rate of discovery of bacterial pathogens dropped (Fournie et al., 2015). The nature of swine production systems undoubtedly played a large role in this emergence. Given the evolving nature of pig production, the requirements for risk-based surveillance and risk management have been identified as priorities as the global level (Fournie et al., 2015). Over the last two years only, two foreign viral pathogens, PED and Porcine Delta CoronaVirus (PDCoV) emerged in North American swine, and surveillance proved to be critical in early identification of cases and disease containment. In Ontario, the primary source of diagnostic data for PED continues to the Animal Health Laboratory (AHL). Subset of diagnostic data from AHL is entered into the Ontario Swine Health Advisory Board's (OSHAB) database, which additionally contains information about herd demographics and secondary cases due to animal movement. The hypothesis is that integration of data from different sources could lead to accurate assessment of trends in PED at the population level, improve detection of new cases, and therefore measure incidence; a fundamental measure that is lacking for many diseases in swine populations.

Methods: The major existing source of data for the proposed research will be disease control programs for PED. Detection of outbreaks in a prospective manner will be conducted using the Farrington and CDC algorithms based on new number of: incidence cases, submissions, and positive submissions. Short-term forecasting of incidence and number of submissions will be conducted using time-series models. Our current work based on influenza data suggests that Random Forest models has the best predictive accuracy for the number of submissions, whereas generalized time series models based on Poisson distribution (glarma) have the best predictive ability for the number of positive submissions on a weekly basis.

**Results:** A reporting system using the R platform will be utilized to produce a pdf report of the current

situation with respect to PEDV. The report will contain visualizations of incidence, prevalence and number of sites that achieved disease freedom; number of submissions and number of positive submissions, alarms when outbreaks are detected, and short-term forecast. Approaches and scripts for real-time access to multiple-sources of surveillance data will be developed. This is invaluable in further development of real-time disease surveillance. In addition, computer codes for ongoing data aggregation and analysis will be developed. The outcome of this process will be ongoing analysis and interpretation of data with minimum effort from analysts.

**Industry Implications:** The proposed system is a novel approach to data integration and analytics that currently does not exist. We will generate mechanism of weekly analysis of existing data, their analysis for multiple purposes, and dissemination to those who need to know.

The system that we will develop here will have ability to access data from different sources in realtime including: (i) OSHAB's database on PED, (ii) AHL records at the aggregated level, and (iii) production records on mortality due to gastrointestinal diseases from a commercial database. Descriptive and inferential statistical and data mining approaches will be included for the purposes of describing trends, detection of aberrations, shortterm predictions, and communication to industry stakeholders. The main benefit is that we will have a short and a comprehensive report of the current PED situation on a weekly basis. This approach could then be adapted for other diseases in swine and beyond. Ultimately, this research will lead to better utilization of swine health data to support optimal health, welfare and productivity of the Ontario swine herd.

#### Reference:

1. Fournié G, Kearsley-Fleet L, Otte J, Pfeiffer DU. Spatiotemporal trends in the discovery of new swine infectious agents. Vet Res. 2015 Sep 28;46:114. doi: 10.1186/s13567-015-0226-8.

#### Prevalence of various serotypes of Streptococcus suis in clinical cases and healthy-carrier pigs

Emily Arndt BSc<sup>1</sup>; Vahab Farzan DVM, MSc, PhD<sup>1</sup>; Glenn Soltes MSc<sup>2</sup>; Janet MacInnes PhD<sup>2</sup>; Robert Friendship DVM, MSc, Dip ABVP<sup>1</sup>

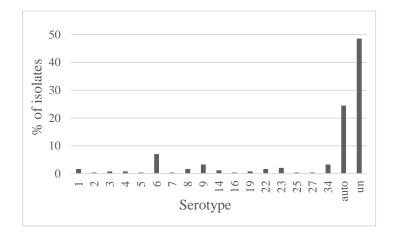
Department of Population Medicine<sup>1</sup>, Department of Pathobiology<sup>2</sup>, University of Guelph

**Introduction:** The Gram-positive bacterium *Streptococcus suis* causes a wide range of diseases in swine (1). Decreased performance and mortality resulting from *S. suis* infection have a significant economic impact on swine production. The objective of this study was to investigate the serotype distribution of *S. suis* isolates recovered from clinical and healthy-carrier pigs, and to compare culture and PCR methods for *S. suis* detection.

**Methods:** Nasal, tonsillar, and vaginal swabs were collected from healthy pigs and tonsillar and meningeal swabs as well as tissue from tonsil and lymph nodes from clinically-ill pigs were obtained from pigs on 17 farms. Duplicate swabs were collected: one for culture and serotyping and the other for testing for the presence of the *gdh* gene by PCR. A logistic regression method with farm as a random effect was used to compare the presence of *S. suis* and its serotypes in diseased and healthy pigs, and among pigs at different stages of production.

**Results:** Of 405 samples, 310 were collected from healthy and 95 from sick pigs. There was no significant difference between the recovery rate of *S*. suis from suckling versus nursery pigs (P = 0.7), or from sows versus finishers (P = 0.9). However, S. suis was more likely to be recovered from suckling and nursery piglets than from sows and finishers (P <0.001). S. suis was recovered more often from healthy pigs as opposed to sick pigs (P < 0.001). However, it is possible that some of the sick pigs tested might have been treated with antibiotics before sample collection. Seventeen serotypes were identified, with type 6 being the most common serotype (Figure 1). However, this type was only isolated from healthy pigs. Nineteen percent and 52% of isolates autoagglutinated or were untypable, respectively; untypable isolates were most likely to be recovered from healthy pigs (P < 0.01). There was no agreement between the culture and PCR methods for S. suis detection.

**Figure 1**: *S. suis* serotypes isolated from healthy and clinically ill pigs



**Conclusions:** More clinical cases are needed to draw conclusions regarding the serotype distributions in sick pigs.

**Industry Implications:** This research will provide valuable information on distribution of *S. suis* serotypes in healthy carrier and sick pigs on Ontario swine farms. This knowledge can be used to ensure that the right strains are chosen for autogenous vaccines, and to help in the design of appropriate management changes to reduce the prevalence of *S. suis* disease outbreaks. It will also help to develop a PCR based method to serotype *S. suis* isolates.

**Acknowledgements:** Thanks to the OMAFRA-University of Guelph Research Partnership Program for funding this project, Gallant Custom Laboratories and the Animal Health Laboratory for their support and technical assistance, as well as Ontario pig producers for their participation in this study.

#### **Reference:**

1. Gottschalk M. *Streptococcal* diseases. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, editors. Diseases of Swine. 10th ed. Oxford, United Kingdom: Blackwell Publ. 2012: 841–855.

#### Kinetics of young porcine jejunal alkaline phosphatase in hydrolyzing endotoxin

Nicole A. Burello BSc; Tania Archbold BSc; Weijun Wang MSc, PhD; Ming Z. Fan MSc, PhD Department of Animal Biosciences, University of Guelph

**Introduction:** Endotoxin lipopolysaccharides (LPS) mediate their toxic inflammatory responses by hosts through binding to toll-like receptors (eg, TLR4) and intestinal apical alkaline phosphatase plays a pivotal role in protecting the gut health through hydrolytic dephosphorylation of the lipid A moiety of LPS (1). Kinetic analyses of intestinal apical alkaline phosphatase hydrolytic activities, including maximum enzyme activity  $(V_{max})$  and enzyme  $(K_m)$ were only conducted chromogenic artificial substrates but not with physiological substrates such as LPS in weanling and growing pigs in our previous studies (2,3). The objectives of this study were to determine time course and kinetics of young porcine jejunal apical alkaline phosphatase for hydrolyzing dephosphorylation of the endotoxin LPS lipid A moiety.

Methods: Jejunal samples were collected from four 10-day old Yorkshire pigs and were pulverized to be homogenous under liquid N<sub>2</sub> (3). Pure LPS, isolated from E. coli 0111:B4, was purchased from Sigma-Aldrich (product number L 302). Time course experiments were conducted by using the lowest LPS concentration (0.125 mg/mL) in incubation media at 37°C, pH 6 and 8, and at 0, 90 and 180 min, respectively. The kinetic experiments were conducted at 37°C, and pH 7.4 for 180 min with LPS concentrations ranging from 0 - 5 mg/mL in incubation media. Free phosphorus (Pi) release was measured by using the PiColorLock detection reagent (Innova Biosciences) (4). Contributions of the non-specific background Pi associated with LPS in the time course and the kinetic experiments were corrected. All enzymatic incubations were conducted with 19 – 27 µg of the homogenized porcine jejunal protein.

**Results:** At the LPS concentration of 0.125 mg/mL in incubation media and at 37°C, the hydrolytic dephosphorylation release of free Pi from the endotoxin LPS lipid A moiety by the young porcine jejunal apical alkaline phosphatase was linear from 0 to 180 min. And the linearity was not significantly affected by media pH (pH at 6:  $y = 0.00369(\pm 3.281e-4)*x$ ,  $R^2 = 0.86$ , P < 0.05, n = 12; vs. pH at 8:

 $v = 0.00394(\pm 4.5561e-4)*x$ ,  $R^2 = 0.72$ , P < 0.05, n = 12). Kinetics of the young porcine jejunal apical alkaline phosphatase for hydrolyzing dephosphorylation of the E. coli 0111:B4 endotoxin LPS lipid A moiety are determined by plotting the mean enzyme activity values averaged from the four piglets with corresponding LPS concentrations (n = 11), including  $V_{max}$  at 0.24 ug Pi/mg protein.min (SE  $\pm$  0.04), P < 0.05; and  $K_m$  at 2.68 mg/mL (SE  $\pm$ 0.81), P < 0.05;  $R^2 = 0.96$ . In comparison,  $K_m$  values for dephosphorylation of LPS lipid A moiety by human alkaline phosphatase isomers were reported to be 0.62 - 0.79 mg/mL (4). Thus, our kinetic results suggest that young pig jejunal alkaline phosphatase is 2-3 fold less efficient in detoxifying endotoxin LPS compared with these enzyme isomers in humans.

**Conclusion:** Results from this study show that young pig jejunal alkaline phosphatase affinity is 2-3 fold less efficient in detoxifying endotoxin LPS in comparison with humans.

**Industry Implications:** This study results suggest that young porcine gut is susceptible to bowel inflammation and infection. Effective dietary strategies need to be developed to help young pigs cope with gut health challenges in commercial swine production.

**Acknowledgements:** The NSERC Discovery Program and the Metagen Enzyme Corporation.

- 1. Fan MZ, Archbold T. Anim Nutr. 2015; 1:138-143.
- 2. Fan MZ *et al.* J Nutr Biochem. 1999; 10:299-305.
- 3. Lackeyram D et al. J Nutr. 2010; 140:461-468.
- 4. Kiffer-Moreira T *et al.* PLOS One 2014; 9(2):e89374.doi:10.1371/journal.pone.00893

#### Fucose as a preservative of bioactive peptide

Emily K Hill BSc<sup>1</sup>; Xiaofeng Sun PhD<sup>1</sup>; Hugh Y Cai DVSc.<sup>2</sup>; CFM de Lange PhD<sup>1</sup>; Julang Li PhD<sup>1</sup> Department of Animal Biosciences, University of Guelph<sup>1</sup>, Animal Health Laboratory, Laboratory Services Division, University of Guelph<sup>2</sup>

Introduction: Fucoidan is a natural polymer present in brown seaweed and is mostly composed of fucose monomers (1). It has been demonstrated to possess beneficial antioxidant, anti-inflammatory, and anti-tumor properties (2). However, the role of fucoidan and fucose in stabilizing bioactive peptides, thus serving as a preservative, has not been explored. In the current study, we sought to examine the preservation ability of fucose using Epidermal Growth Factor (EGF), a cell growth stimulator and candidate bioactive peptide for inclusion in swine feed.

Methods: Porcine EGF was generated via fermentation using genetically modified yeast. After freeze drying, recombinant EGF was stored in either standard or vacuum sealed packaging and its activity was tested monthly by a cellular proliferation assay using human embryonic kidney (HEK) 293 cells. Each sample package-type was stored with or without fucose at 4°C, room temperature (RT), or 28°C. The presence of EGF in each sample was confirmed by Western Blot. Bacteria levels in stored EGF +/- fucose samples were evaluated via colony forming (CFU) quantitation. unit Characterization of contaminate bacteria isolates was achieved using MALDI-TOF spectrometry. Protease activity was measured for EGF +/- fucose samples stored at 28°C for one month (30 days).

**Results:** In the absence of fucose, cellular proliferation results indicated a loss of bioactive function for EGF stored at room temperature in standard seal packaging after 2 months. When stored at 28°C, EGF failed to promote cellular proliferation after only a month. Diminishing levels of EGF over this time period were revealed via Western Blot analysis, pointing to a degradation of EGF under these storage conditions. The addition of fucose to the recombinant EGF storage was found to prevent

EGF degradation and maintained its bioactive properties for at least 8 months when stored at both at room temperature and 28°C. Additionally, the fucose-mediated enhancement of EGF shelf life was shown to be associated with the inhibition of bacterial growth during storage. Further studies revealed that the isolated bacterial storage contaminants were all of the *Bacillus* genus and were predominantly found to be *Bacillus subtilis*. Storage of EGF in the presence of fucose at 28°C for one month reduced bacterial contamination approximately nine-fold and curtailed protease activity at a level that is comparable to the negative control.

Conclusions: Fucose protects bioactive peptides, such as EGF from degradation. This protective effect may be due to its inhibition of growth of the naturally occurring contaminant bacteria in the stored products. With a decreased bacterial load, fewer proteases can be secreted into storage packaging, ultimately protecting against the degradation of stored bioactive peptides. In addition, our study also showed that fucose increases the shelf life of dry-stored bioactive peptides regardless of temperature and packaging type.

**Industry Implications:** Swine feed can be supplemented with bioactive proteins to enhance nutrient value, production growth rates, and increase profitability (3). The utilization of fucose to preserve these feed additives may expand their shelf life, reducing production costs by preventing the breakdown of these products over time.

**Acknowledgements:** Funding supported by ABvista, NSERC, and OMAFRA, Swine Cluster

- 1. Bilan MI, *et al.* Carbohydr Res. 2010; 345:2038-2047.
- 2. Lim SJ, *et al.* Food Hydrocolloids. 2014; 42:280-288.
- 3. Jacela JY, *et al.* J Swine Health Prod. 2009; 17:270-275.

## An investigation into the relationship between influenza A virus (IAV) and *Streptococcus suis* infections in nursery pigs

<u>Danielle C Hopkins</u> BSc; Zvonimir Poljak DVM, MSc, PhD; Vahab Farzan DVM, MSc, PhD; Robert M Friendship DVM, MSc, DipABVP; Helena Grgić DVM, MSc, PhD Department of Population Medicine, University of Guelph

**Introduction:** *Streptococcus suis* is a common isolate from the tonsils of healthy pigs and under certain circumstances is the cause of sporadic disease outbreaks, most often occurring in nursery pigs (2). Factors that influence transition from tonsillar colonization to clinical disease are poorly understood. It has been shown in-vitro that adherence and virulence of S. suis increases when swine epithelial cells were pre-infected with classical H1N1 influenza virus (1). Influenza A virus (IAV) is also a common post-weaning pathogen. The objectives of this study are to determine if infection with IAV predisposes piglets to develop clinical signs of S. suis infection, and to determine if pigs that develop clinical S. suis disease have defective innate immunity.

**Methods** The interaction will be analyzed using a case-control study design. Five farms will be enrolled in the study. The samples will be collected from 10-12 cases per farm. Cases will be selected if pigs are demonstrating clinical signs for acute meningitis caused by S. suis infection and controls will be healthy age-matched pen mates. Post-mortem examinations will be conducted on the cases and meningeal swabs collected, as well as lung samples for virology and histology investigation. Nasal and tonsillar swabs as well as blood samples will be taken from both cases and controls. Bacterial swabs will undergo culturing for S. suis, DNA extractions, and PCR analysis. The isolates will also be serotyped using co-agglutination. The blood samples will be tested using ELISA and HI inhibition for the presence of different classes of antibody (IgA and IgM) against IAV to determine if there was recent and/or historical IAV infection. An ear notch will be collected from

cases and controls and used to identify potential genetic markers associated with innate immunity to *S. suis* and IAV. The cases and controls will be compared to determine if recent infection with IAV is associated with systemic infection with *S. suis*, which could elucidate pathogenesis of *S. suis* infection

**Preliminary Results:** Data are still being collected. On one farm, over a 3-week period, samples were collected from 12 cases of *S. suis* meningitis and an equal number of controls. The bacterial swabs from the tonsils and meninges of cases were cultured, and when S. suis was isolated, DNA was extracted and PCR performed to confirm S. suis. Ten of 12 cases were positive for S. suis based on the meningeal swabs. In addition, there was evidence of lung lesions based on gross examination for 3 out of the 10 positive cases. Virology test results for all submissions are pending. Additional farms will be included to obtain information on different serotype of S. suis, IAV and husbandry factors that could influence the relationship between the two pathogens.

**Industry Implications:** If an interaction between these two pathogens exists, then different vaccination protocols and preventative measures could be implemented in order to decrease morbidity and mortality rates.

**Acknowledgements:** Swine Improvement Porc, OMAFRA UoG Research Partnership

- 1. Wang Y, *et al*. Infection and Immunity, 2013;81:4498-4508.
- 2. Gottschalk M. Diseases of Swine 10<sup>th</sup> Edition. 2012;841-853.

#### Dietary ammonia appearance in portal blood of pigs fed diets deficient in non-essential amino acid nitrogen is incomplete

W.D. Mansilla MSc<sup>1</sup>; A.K. Agyekum MSc<sup>2</sup>; C.L. Zhu MSc<sup>1</sup>; J.K. Htoo PhD<sup>3</sup>; C.M. Nyachoti PhD<sup>2</sup>; C.F.M. de Lange PhD<sup>1</sup>

Department of Animal Biosciences, University Guelph<sup>1</sup>
Department of Animal Science, University of Manitoba<sup>2</sup>
Evonik Nutrition & Care GmbH, 63457, Hanau-Wolfgang, Germany<sup>3</sup>

Introduction: Inclusion of crystalline amino acids (AA) allows formulation of diets that better match the animal's AA requirements while decreasing dietary protein content. Diets that are highly supplemented with crystalline AA, however, may be deficient in total nitrogen (N) for endogenous synthesis of non-essential AA (NEAA). Previous studies in our lab have shown that dietary ammonia, in the form of ammonium citrate, appears an effective source of N for endogenous synthesis of NEAA. The objective of the present study was to further understand dietary ammonia absorption and ammonia-N metabolism in the portal drained visceral organs (PVDO) and liver of growing pigs.

Methods: Four gilts of about 24 kg were fitted with 4 catheters (i.e., carotid artery, and portal, hepatic and mesenteric veins; 1) for serial blood sampling. A purified basal diet was formulated to be deficient in NEAA-N, but to meet requirements for all essential AA (EAA; 2). Cornstarch (Control) or ammonium citrate (Ammonia) was added to the basal diet to maintain or supply 2.72 % extra crude protein, respectively. Pigs were fed restricted at 2.8 x maintenance ME requirements in 3 equal meals every 8 h for 7 d. On the last day, a saline solution of ρ-amino hippuric acid (PAH) was infused continuously to determine blood flow in the portal and hepatic veins. Blood samples were taken at 0, 30, 60, 90, 120, 180, 240, 300, 390 and 480 min after feeding, and blood plasma was analysed for ammonia, urea-N, and PAH concentration. Pigs were assigned to the dietary treatments following a cross-over design.

Plasma blood flow in portal and hepatic veins were calculated considering the dilution of PAH in blood compared to the infusate. Hepatic artery plasma flow was calculated as the difference between hepatic and portal vein blood flows. Metabolites (e.g., urea or ammonia-N) input or output was calculated from blood flow to or from the PVDO or liver, and corresponding metabolite concentrations. The balance of specific metabolites in either the PVDO or the liver was calculated by subtracting output from input.

**Results:** One observation per treatment was lost due to incomplete feed intake before blood sampling was started. For Ammonia treatment, ammonia-N balance in the PVDO increased during the first 3 h after feeding. At 480 min after feeding, values became similar to time 0 min for both treatments. Ammonia-N balance in the liver decreased at 30 min for Ammonia compared to Control and remained lower until 3 h after feeding. At the end of the sampling period, ammonia-N balance in the liver returned to similar values compared to time 0 min for both treatments.

Urea-N balance in PVDO was not different between Control and Ammonia, indicating that the intestinal wall is not using dietary ammonia-N for urea synthesis. Total urea-N balance in the liver increased for Ammonia compared to the Control. Ammonia-fed pigs consumed ~1720 mg of ammonia-N per meal but only 410 mg (~24 %) of ammonia-N was recovered in the portal vein per

ammonia-N per meal but only 410 mg (~24 %) of ammonia-N was recovered in the portal vein per feeding period. This implies that the intestinal wall is using ammonia-N for the synthesis of nitrogenous metabolites other than urea (e.g., NEAA including citrulline).

**Conclusion:** Appearance of dietary ammonia occurs within 30 min after feeding. The intestinal wall may use ammonia-N for the synthesis of NEAA as its appearance in the portal vein is incomplete.

**Industry Implications:** When diets are highly supplemented with crystalline AA, ammonia-N may be used to provide extra N for the synthesis of NEAA. Further understanding of PDVO role in dietary ammonia-N metabolism is warranted.

**Acknowledgments:** Funding provided by Evonik Industries, Swine Innovation porc and OMAFRA.

- 1. Rerat, A., et al. Bri J Nutr. 1991;68:111-138.
- 2. National Research Council (NRC). 2012. Washington, DC.
- 3. Haussinger, D. Eur J Biochem. 1983;133:269-275.

#### Growth performance and carcass quality in pigs fed a low or high complexity nursery diet

<u>Heather Reinhardt</u> BSc<sup>1</sup>; Vahab Farzan DVM, MSc, PhD<sup>2</sup>; C.F.M. de Lange PhD<sup>1</sup> Department of Animal Biosciences<sup>1</sup>, Department of Population Medicine<sup>2</sup> University of Guelph

Introduction: Feed is the main cost of pork production. A previous study showed that feeding pigs less complex and less expensive nursery diets will compromise growth performance during the nursery phase, but may induce compensatory growth thereafter and reduce production costs (1). The objective of this study was to investigate effects of using a low complexity (LC; containing reduced animal protein) nursery pig feeding program on subsequent growth performance and carcass quality under commercial farming conditions.

Methods: The study was conducted on 8 commercial farms; on each farm 60 piglets were selected from 8-10 sows at 24-96 hours after birth. Pigs were weighed five times: at birth, at weaning, at the end of nursery, grower and finisher phases. The study was conducted twice on each farm. At weaning, the pigs were assigned to one of two dietary treatments: LC vs. high complexity (HC) nursery feeding program. Carcass quality information was collected for all pigs at slaughter. A survey was conducted to determine pig density, pig flow, in-feed medication, vaccination and diseases history. A multilevel mixed-effect linear regression modeling method was used to analyze average daily gain, body weight, and carcass quality.

**Results:** A total of 832 pigs were enrolled in the study. The mean average daily gains and body weight of pigs fed HC versus LC nursery feeing programs are shown in Table 1. Mean back fat depth (mm), loin eye depth (mm), and lean yield (%) were 20.4, 68.4 and 60.3 for HC and 20.8, 67.6 and 60.2 for LC, respectively. Multivariable analysis with farm as random effect suggested that average daily gain of pigs on LC was not different from that of pigs on HC (P>0.05). However, mean age at slaughter for HC (175.5 days) was higher than that for LC (173.8 days; P=0.04). Mixed-level regression

analysis with farm and pig as random effect showed that there was no effect of dietary treatment on body weight of pigs at all phases and on carcass characteristics (*P*>0.05).

**Table 1**: Mean average daily gain (ADG, kg) and body weight (BW, kg) in pigs fed LC or HC nursery feeding programs.

	Suckling	Nursery	Grower	Finisher
<u>HC</u>				
ADG	0.244	0.428	0.828	1.036
BW	7.1	24.3	60.6	105.1
<u>LC</u>				
ADG	0.239	0.417	0.836	1.042
BW	6.9	23.8	60.2	104.7

Conclusions: The use of a LC nursery feed program in a commercial setting is shown to have no negative impacts on pig growth and performance up to slaughter weight. Interestingly, the pigs fed a LC diet appeared to have compensatory growth before the end of the nursery phase while in the previous experimental trials complete compensatory growth was not observed until the grower phase (1).

**Industry Implications:** By feeding a LC nursery feeding program and inducing compensatory growth in the nursery; the cost of swine production could be reduced, resulting in an increased profitability for producers without compromising performance.

**Acknowledgments:** We would like to thank Swine Innovation Porc, Ontario Pork, OMAFRA UoG Research Program, and NSERC-CRD for financial support, as well as Synergy Swine Inc, Conestoga Meat Packers and all participating producers.

#### **Reference:**

1. Skinner LD, et al. J. Anim. Sci. 2014; 92:1044-1054.

#### A study of Salmonella shedding in pigs from early life until slaughter

Margaret H Ainslie BSc<sup>1</sup>; Vahab Farzan DVM, MSc, PhD<sup>2</sup>; Robert M Friendship DVM, MSc, Dip ABVP<sup>2</sup>; Cornelis FM de Lange, PhD<sup>3</sup>; Brandon NM Lillie DVM, PhD, Dip ACVP<sup>1</sup> Departments of Pathobiology<sup>1</sup>, Population Medicine<sup>2</sup>, Animal Biosciences<sup>3</sup>; University of Guelph

**Introduction:** Salmonella is a foodborne pathogen affecting pigs and humans, with an estimated 90 000 human cases annually in Canada (1). Better knowledge of Salmonella shedding over the pig's lifetime is needed in order to develop more effective control programs on swine farms. The objective of this study is to investigate Salmonella shedding and colonization in pigs from birth to slaughter.

Methods: Eight farms were included in the study. On six farms a winter and summer cohort, and on two farms only a summer cohort were monitored. For each cohort 60 piglets were selected from eight to ten sows within 96 hours of farrowing. During the nursery stage, pigs received either a high complexity or low complexity diet (containing reduced animal protein). Fecal samples or rectal swabs were collected within 96 hours after birth (only in the winter cohort), at weaning, and at the end of nursery, grower and finisher stages. Tissue samples were collected at slaughter. All samples were cultured for Salmonella. A multi-level mixed-effects modelling method was used to analyze Salmonella shedding at different stages of production as well as among pigs in the two different nursery diet groups. The Spearman's rank correlation coefficient was used to determine the association between Salmonella shedding and its presence in tissue samples.

**Results:** Prevalence of *Salmonella* shedding in pigs during the summer cohort and its presence in tissue samples is shown in Table 1. In the winter cohort, to date *Salmonella* was detected in 4.9% (20/409) of piglets within 96 hours of farrowing, 12.4% (43/347) of pigs at weaning, 12.7% (35/276) at end of nursery, 7.2% (19/266) at end of grower, and 28.5% (47/165) of pigs at end of finisher. *Salmonella* was cultured from 23.2% (76/328) of tonsils and 29.1% (60/206) of lymph nodes but presence of *Salmonella* in tissue samples was not correlated with fecal shedding (P>0.05). Multilevel mixed-effects analysis of data collected to date indicates an increase in prevalence of *Salmonella* shedding from early life to the finisher period (P<0.05) with no effect of dietary

treatment on *Salmonella* shedding and colonization (P>0.05).

**Table 1:** Percent of pigs tested positive for *Salmonella* shedding and for presence of *Salmonella* in tissue samples (summer cohort)

Farm	Weaning	Nursery	Grower	Finisher	Tonsil	Lymph nodes
A	0.0	0.0	0.0	0.0	0.0	
В	1.8	3.6	0.0			
C	5.4	32.0	16.0	26.0	23.0	26.0
D	8.7	0.0	18.0	78.0	59.0	53.0
E	30.0	5.3	0.0	0.0	10.0	4.0
F	12.0	7.0	5.5	3.7	16.0	39.0
G	6.3	66.0	7.3	48.0	60.0	64.0
H	3.4	5.6	5.2	0.0	10.0	2.5
Total	8.9	14.2	5.9	25.9	26.1	29.1

**Conclusions:** These results indicate that *Salmonella* shedding peaks near the end of the finishing period and remains high at slaughter. However, detection of *Salmonella* in finisher pigs may not be a good indicator for presence of *Salmonella* at slaughter.

**Industry Implications:** These results may help to understand the stages of production which are most important for control of *Salmonella*. Reduced salmonellosis associated with pork production will benefit public health, while the pork industry should experience improved public perception, herd health, animal welfare and profitability.

**Acknowledgements:** We would like to thank OMAFRA (Food safety research program, HQP Scholarship program, and U of G Partnership), Swine Innovation Porc, and Ontario Pork for financial support as well as participating pork producers and Conestoga Meat Packers.

**References:** (1) Thomas et al. (2013) Foodborne Pathog Dis 10(7) 639-48.

## Fermentation of soybean meal using newly isolated *Bacillus amyloliquefaciens* to improve its nutritional value

N. Akhtar PhD¹; S. Medeiros MSc¹, H. Y. Cai DVM, DVSc², C.F.M. de Lange PhD¹; J. Li PhD¹\*

Department of Animal Biosciences¹, Animal Health Laboratory², University of Guelph

**Introduction:** Soybean meal is a commonly used protein source for animal feed. However, it contains anti-nutritional factors such as allergens, phytate, oligosaccharides and fibers such as cellulose, which limit its utilization. Microbial fermentation using bacteria or fungi has the capability to improve the nutritional value of soybean meal by altering the native composition. Our previous experiments, using smaller scale solid state fermentation, showed that fermentation resulted in degradation of large molecular weight allergenic proteins, an increase in amount of small-sized peptides, and improvement in both essential and non-essential amino acid contents. The objective of the current study was to optimize the condition for larger-scale fermentation and to investigate if the fermentation can also decrease other anti-nutritional factor, such as cellulose and oligosaccharides in soybean meal.

**Methods:** Seven (07) bacterial strains were isolated and identified using 16S rRNA gene sequence analysis from fermented food and intestines of grass carp. All the isolates were screened for cellulolytic and xylanolytic activity using the 3, 5, dinitrosalicylic acid (DNS) method. An efficient strain (isolate-4) was used for fermentation of soybean meal and fermentation conditions were optimized. The fermented soybean meal was analyzed for protein profile, as well as nutrient composition and allergenicity.

**Results:** Carboxymethyl cellulase, filter paperase,  $\beta$ glucosidase and xylanase activity analyses revealed that all the seven bacterial strains isolated from fermented food and grass carp process activities of these four enzymes. Among them, isolate-4 identified as Bacillus amyloliquefaciens from fermented food showed significant activity for carboxymethyl cellulase, filterpaperase, glucosidase and xylanase. It was therefore chosen for further fermentation studies. Compared to previous fermentation procedures, we found that the fermented sovbean meal showed improved protein profile when pre-soaking the soybean meal in water (soybean meal:water, 1:1) for 4 h, followed by 32 h of fermentation at 37°C after inoculation.

Conclusions: Solid state fermentation using food grade bacteria processing proteolytic, cellulolytic and xylanolytic activity may be a cost effective mean to decrease allergen and anti-nutritional factor level, improve protein profile of soybean meal. Further study should reveal its efficiency in decreasing cellulose, oligosaccharides level in soybean meal. Scaling-up of this fermentation process with relatively low moisture and high substrate loading is expected to make it economically feasible for industrial implications.

**Industry Implications:** Unprocessed soybean meal contains several allergenic proteins and other antinutritional factors, limits it's feeding to the early weaned piglets. Microbial fermentation of soybean meal may be an effective approach for decreasing these undesirable factors for soybean meal applications in the swine industry.

**Acknowledgments:** This project has been supported by Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA).

## An investigation of an outbreak of *Streptococcus suis* infection in nursery pigs and evaluation of vaccine efficacy

<u>Danielle Hopkins</u> BSc; Zvonimir Poljak DVM, MSc, PhD; Vahab Farzan DVM, MSc, PhD; Robert Friendship DVM, MSc, DipABVP

Department of Population Medicine, University of Guelph

**Introduction:** *Streptococcus suis* has 35 serotypes that may be present as commensal bacteria or opportunistic pathogens in the nasal cavity and tonsils of the majority of pigs (1). The occurrence of S. suis clinical infections in swine herds is usually characterized by a low incidence (3-5%) of clinical cases that show signs consistent with septicemia, and neurological involvement (1). Treatment typically involves injection with penicillin or ampicillin if caught in the early stages of disease progression, however, even with early treatment prognosis is often poor (3). Autogenous vaccinations are available as a preventative measure to help control the morbidity and mortality rate caused by S. suis but their overall efficacy is not widely accepted (3). Occasionally, S. suis causes major outbreaks affecting up to 30% of pigs in the nursery, but factors contributing to development of clinical cases are poorly understood (2). In this study the production records from a 300-sow farrow tofinish herd that experienced an outbreak of S. suis-related mortality were examined. The objectives of the study were 1) to investigate sowand litter-level factors that were associated with the hazard of dying during the nursery phase; 2) to evaluate the direct efficacy of an autogeneous S. suis vaccine.

**Methods:** The production records from a 300-sow farrow to-finish herd experiencing an outbreak of *S. suis*-related mortality were examined. The farm-level data factors considered were: sow parity, litter size at birth and weaning, and date of weaning and mortality. To evaluate the vaccine efficacy, 4 weaning cohorts were included in the trial with 25% and 75% of piglets within a litter randomly allocated to the non-vaccinated and vaccinated group, respectively. A Cox's proportional hazard model was used to

evaluate both risk factors and direct vaccine efficacy.

**Preliminary Results:** Preliminary analysis indicates that <50% of mortality within litters occurred during the 3<sup>rd</sup> or 4<sup>th</sup> week in the nursery. During the outbreak of *S. suis* mortality within the nursery was between 20-30%, greatly exceeding the normal mortality levels typically attributed to *S. suis* (3-5%). During the vaccination trial sporadic outbreaks of *S. suis* resulted in mortality reaching more than 20% in the 5 cohorts involved in the vaccination trial. This is consistent with the current literature surrounding autogenous vaccines (2)

**Conclusions:** Further analysis of the data needs to be done, and more research into the risk factors and patterns of *S. suis* outbreaks are needed to be preformed.

**Industry Implications:** This study may help to determine litter-level risk factors for *S. suis* clinical disease and may provide information to help veterinarians design more effective *S. suis* control and prevention strategies. This study could also lead to enhancing our knowledge surrounding the use and efficacy of *S. suis* vaccination protocols.

**Acknowledgements:** Swine Improvement Porc, and University of Guelph/OMAFRA Research Partnership

- 1. Gottschalk M. Diseases of Swine 10<sup>th</sup> Edition. 2012;841-853.
- 2. Reams RY, *et al.* J Vet Diagn Invest. 1994;6:326-334.
- 3. Torremorell M, *et al*. J Swine Health Prod. 1997;5:139-143.

## Salmonella shedding and antimicrobial resistance in naturally infected pigs treated with in-feed flavophospholipol

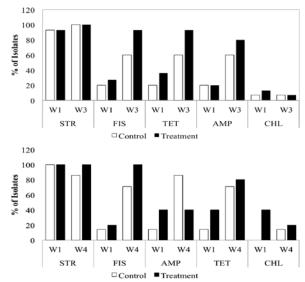
Saranya Nair BSc; Vahab Farzan DVM, MSc, PhD; Terri L O'Sullivan DVM, PhD; Robert M Friendship DVM, MSc, Dip ABVP Department of Population Medicine, University of Guelph

Introduction: The presence of multi-drug resistant Salmonella in the swine population is an important concern. Research suggests food safety flavophospholipol, an antibiotic with nontransferable resistance, can alter the gut microflora in favor of beneficial bacteria inhibiting the colonization of (1).Furthermore. Salmonella in animals flavophospholipol by inhibiting the growth of R-plasmids carrying bacteria, in a process referred to plasmid-curing effect (1), may reduce antimicrobial resistance (AMR) (2). The objective of this study is to investigate whether in-feed flavophospholipol can reduce Salmonella shedding and antimicrobial resistance in naturally infected growerfinisher pigs.

**Methods:** Naturally infected 9-week-old pigs (n=45) were purchased from a farm with a history of salmonellosis and were housed at the Ponsonby Animal Research Facility (University of Guelph). Treatment (n=25) pigs were given 4 ppm of in-feed flavophospholipol (Flavomycin<sup>®</sup>, Huvepharma AD) and control (n=20) pigs were fed an identical but nonmedicated feed. Individual weekly fecal samples were collected for 10 weeks and cultured for Salmonella. Salmonella isolates recovered before treatment (Week 1) and after treatment (Week 3 & 4; weeks with the highest Salmonella prevalence) were tested for antimicrobial susceptibility using a broth microdilution method. Statistical analysis was conducted using STATA 14.1. A multivariable logistic regression method was used to compare Salmonella shedding and antimicrobial resistance amongst the treatment groups.

**Results:** The mean prevalence of *Salmonella* shedding over 10 weeks in pigs that received flavophospholipol (27%) was not significantly different from that in the control pigs (28%) (*P*>0.05). Antimicrobial susceptibility testing revealed an increase in resistance over time for ampicillin (AMP), chloramphenicol (CHL), streptomycin (STR), sulfisoxazole (FIS), and tetracycline (TET) of the 12 antimicrobials tested. *Salmonella* isolates from pigs in the flavophospholipol group had similar resistance patterns as isolates from

the control group after 3 and 4 weeks of the trial (P>0.05) (Figure 1).



**Figure 1:** AMR in *Salmonella* isolates recovered from pigs before treatment (Week 1) and after treatment (Week 3 & 4)

**Conclusions:** Pigs given 4 ppm of in-feed flavophospholipol continued to shed *Salmonella* with similar antimicrobial resistance to non-medicated pigs. Future studies are needed to investigate the preventive effect of flavophospholipol if administered before pigs become infected with *Salmonella*.

**Industry Implications:** The findings suggest that farmers with a *Salmonella* problem in the grower barn may need to start feeding flavophospholipol to pigs at earlier stage, i.e. nursery stage to stop the bacterial shedding and reduce antimicrobial resistance.

**Acknowledgements:** OMAFRA-FSRP, OMAFRA-UofG Research Partnership and Huvepharma.

- 1. van den Bogaard *et al.* Antimicrobial Agents and Chemotherapy. 2002; 46(1):110-118.
- 2. Dealy J & Moelle M. Journal of Animal Science. 1976; 42:1331-1336.

#### Pharmacokinetics and toxicity of 1.0mg/kg meloxicam in neonatal piglets

<u>Abbie V Viscardi</u> BSc; Patricia V Turner MS, DVM, DVSc Department of Pathobiology, University of Guelph

Introduction: Meloxicam is a nonsteroidal, antiinflammatory drug licensed for use in food animals in North America. The current labelled dose for pigs is 0.4mg/kg, determined from studies with sows and grower pigs experiencing chronic inflammatory pain (1). However, recent evidence suggests this dose may not be efficacious when provided to piglets experiencing acute pain (for example, after castration and tail docking) and a higher dose of 1.0mg/kg may be more appropriate (2,3). The pharmacokinetics of meloxicam have not been studied in piglets of the age when they are routinely processed (<10 days old), nor have the possible toxicity effects been evaluated for this increased dose. These are important to ensure drug safety. The objective of this study was to determine the pharmacokinetics and possible toxicity meloxicam at 1.0mg/kg, when given intramuscularly to 8-day old piglets.

**Methods:** Piglets were habituated to handling 4 days prior to trial start using short, positive handling sessions, 3x per day. Preceding drug administration, 5 piglets (3 male, 2 female) were anesthetized with isoflurane/O<sub>2</sub> and percutaneously instrumented with a jugular catheter for blood collection, which was flushed with heparin/saline. 24h after surgery, piglets (n=12, 5 of which had catheters) were given a single I.M. injection of 1.0mg/kg meloxicam. 1.0mL of blood was collected from the catheters at 0, 0.5, 1, 2, 4, 8, 12, 24, 36 and 48h post-injection and plasma was separated and sent to Iowa State University for meloxicam concentration analyses. Piglets were euthanized 48h after meloxicam injection, gross examinations were conducted and samples of stomach, liver and kidney were collected for histologic evaluation.

**Results:** A total of 44 plasma samples were analyzed using liquid chromatography-mass

spectrometry to determine blood drug levels. The results were averaged at each time point across all piglets (n=5). The time to maximum blood drug concentration (Tmax) was 0.5h at (Cmax) 3.122ug/mL. Additional pharmacokinetic information will be provided in the poster. There was no evidence of toxicity associated with using 1.0mg/kg meloxicam after gross and histological evaluation of samples from potential target organs.

**Conclusions:** 1.0mg/kg meloxicam I.M. is safe to administer to piglets. Future studies will look at whether it is more effective at reducing acute pain in piglets than the labelled dose of 0.4mg/kg.

**Industry Implications**: Producers will soon be required to administer analgesia prior to castration and tail docking of piglets (July 2016, NFACC), so it's important that safe and efficacious doses of analgesics be identified.

**Acknowledgements:** Funding provided by National Pork & Ontario Pork

- 1. Hirsch AC, et al. J. vet. Pharmacol. 2003;26:355-360.
- 2. Fosse TK, et al. J. vet. Pharm. Therap. 2008:31:246-252.
- 3. Reyes L, et al. 2002. Pharm Biochem Behav. 2002;73:521-528.

## Impact of feeding partly fermented DDGS on growth performance and gut microbiome of nursery pigs

M. Wiseman MSc<sup>1</sup>; A. Khafipour, MSc<sup>2</sup>; E. Khafipour, MSc, PhD<sup>3</sup>; S. Hooda<sup>1</sup> DVM, MSc, PhD<sup>1</sup>; D. Wey BSc<sup>1</sup>; C.F.M. de Lange; MSc, PhD<sup>1</sup>.

Department of Animal Biosciences, University of Guelph<sup>1</sup>, Department of Immunology, University of Manitoba<sup>2</sup>,

Department of Animal Science, University of Manitoba<sup>3</sup>

**Introduction:** diets Feeding with a high insoluble:soluble fiber ratio has been suggested as a strategy to improve gut function in newly weaned piglets through decreased digesta viscosity, reduced gut fill and lower pathogen and total bacterial growth (1). Soluble fiber content can be reduced through partial fermentation of co-products with enzymes and microbial inoculants, while also increasing organic acid and probiotic content of liquid fed diets. This study was carried out to determine the effects of partial fermentation of corn dried distillers grains with solubles (DDGS), which has a naturally high insoluble:soluble fiber ratio, on growth performance and the gut microbiome of nursery pigs fed liquid diets containing DDGS.

Methods: DDGS containing liquid diets were fed to newly weaned pigs that had either high or low body weight at weaning (HBWi vs LBWi; 4 pens/diet and BWi; 14 pigs/pen). Enzymes (67.2 IU β-glucanase and 51.4 IU Xylanase/g DDGS; AB Vista) and silage inoculant (360,000 CFU Pediocoocus pentosaceus 12455 and Propionibacterium jensenii 30081/g DDGS, Lallemand Inc) were added to dry DDGS at the time of liquid feed preparation and delivery (treatment UNFER) or allowed to ferment with DDGS (1 to 7 days at 40°C; 16% dry matter; treatment **FER**). Diets were composed of a common base supplement (corn and soybean meal based) for each of three phases (P; day 0-7, 7-20, 20-48), mixed with DDGS (7.5 (P1), 16.25 (P2) and 25 (P3) % of diet dry matter) and water (to 25% diet dry matter). Per pen dry matter intake (DMI) and individual pig BW's were determined each week for calculating daily BW gain (ADG). On d42 postweaning, ileal digesta, ileal mucosa, and fecal samples were collected from 2 pigs/pen and pooled for bacterial DNA extraction and bacterial profiling.

**Results:** Overall there were no treatment effects (*P*>0.10) on ADG (424 vs. 424±14 g/d for HBWi and 404±15 vs. 386±12 g/d for LBWi) and DMI (605 vs. 581±16 g/d for HBWi and 540±19 vs.

509±16 g/d for LBWi). For d42-48, LBWi pigs on FER had greater ADG (941±60 vs. 773±52 g/d, P<0.05). Variable dietary effects on the microbiome were observed according to sampling location. Alpha diversity of the ileal mucosa and fecal microbiome was lower in pigs on FER (P<0.05), but not affected in the ileal digesta (P>0.10). Pigs on UNFER had enriched Firmicutes populations in the ileal digesta and mucosa, with butyrate producing genera enriched in the mucosa. Conversely, pigs on FER had enriched fecal populations of the probiotic containing class Clostridia. Pathogenic bacteria appeared to be enriched in the feces of pigs on FER (Slackia and Vibrio and ileal digesta of pigs on UNFER (Acinetobacter and Pseudomonas).

Conclusions: Feeding FER DDGS resulted in improved growth performance of LBWi pigs late in the nursery period, suggesting improved nutrient availability and gut function. However, reduced diversity in the ileal mucosa and fecal microbiome and increased populations of potentially pathogenic bacterial populations suggests that pigs fed FER DDGS are more susceptible to enteric disease. It may be beneficial to maintain some soluble fiber (e.g. prebiotic) in nursery pig diets.

**Industry Implications:** Partial fermentation of high-fiber co-products included in nursery pig liquid diets can be used to improve growth performance. However, this strategy may be most useful in co-products with a low initial insoluble:soluble fiber ratio so as to maintain a healthy gut microbiome that is more resistant to enteric disease.

**Acknowledgements:** Funding was provided by OMAFRA, NSERC, and industrial partners of the swine liquid feeding association (www.slfa.ca).

#### **Reference:**

1. Molist F, *et al*. Anim Feed Sci Technol. 2014; 189: 1-10.