Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Dairy and Swine
Research and Development Centre

Centre de recherche et de développement
sur le bovin laitier et le porc

Let's Talk Science at the DSRDC!

First Symposium of the Dairy and Swine Research and Development Centre

Summary of Presentations

June 2011

Canada

Let's Talk Science at the DSRDC!

1st animal science symposium of the Dairy and Swine Research and Development Centre

June 7, 2011

Cat. No. A52-187/2011E-PDF
ISBN 978-1-100-18866-9
AAFC Publication No. 11484E

Aussi disponible en français sous le titre :
Parlons science au CRDBLP! 1er symposium du Centre de recherche et de développement sur le bovin laitier et le porc

Table of Contents

SECTION 1 Introduction to the DSRDC's Animal Science Symposium

The Dairy and Swine Research and Development Centre's annual symposium is an event that is intended to bring together the Centre's multidisciplinary teams and give them a chance to describe the progress of their animal science research.

The scientific research teams at the Dairy and Swine Research and Development Centre (DSRDC) are working on innovative solutions to support the profitability and sustainability of Canadian farms. With its primary focus on dairy cattle, swine and the environment, the Centre has research groups with a wide range of expertise, covering nutrition, forage quality, meat quality, agricultural engineering, microbial ecology, functional genomics, immunology, endocrinology, metabolism, and animal behaviour and welfare. The Centre uses its expertise to find innovative solutions to ensure Canadian farm operations are cost-effective and sustainable.

The DSRDC is proud to launch its first annual animal science symposium.

Let's Talk Science at the DSRDC!

SECTION 2 About the DSRDC

Background

The Dairy and Swine Research and Development Centre (DSRDC) is located in Sherbrooke in the heart of an agricultural region characterized by a large diversity of livestock farms, primarily dairy and swine operations. The Centre opened in 1914 and is currently the only Agriculture and Agri-Food Canada research institute that deals with various aspects of dairy, swine and beef production. The research teams, which combine a number of different disciplines, including the environment, production, nutrition, animal behaviour and functional genomics, conduct both fundamental and applied research to gain insight into and develop solutions to issues related to livestock production. The quality of our scientific expertise is a key asset for the Canadian economy.

In the medium and long term, the future of agriculture, both in Canada and around the world, calls for better management and conservation of natural resources, specifically soils, air, water and biodiversity. According to a report published by the United Nations Food and Agriculture Organization (FAO), world demand for dairy products and meat is expected to double between now and 2050. It is therefore important to develop innovative practices in order to reduce the ecological footprint of livestock operations. If Canada is to play a leading role in sustainable agriculture, we need to develop technologies for small and large farms alike and to design simple, cost-effective management practices.

Vision

To develop technological innovations and know-how to permit the cost-effective production of high-quality milk and meat while ensuring livestock health and welfare and minimizing environmental impacts.

Mission

To provide dairy, beef, swine and sheep producers with technological innovations and know-how permitting the cost-effective production of high-quality milk and meat while ensuring livestock health and welfare and minimizing environmental impacts.

Areas of research

The research centre's strategic mission focuses on the following three areas of research:

Area 1. Livestock production with minimum environmental impact;

Area 2. Livestock health and welfare and socially acceptable agricultural practices;

Area 3. Sustainable dairy and swine production.

Staff

The Centre has a staff of 132 people. Ninety-two employees (professional, technical support and animal care staff) collaborate with the 22 researchers on a variety of research projects. Eighteen people provide support services for operations, thereby facilitating the work of all staff members. On account of its expertise, the Centre attracts about 10 international guest researchers and more than 50 college and university students every year to supplement its core group.

Scientific expertise

Environment: This group consists of three researchers, including two with training in agricultural engineering and one with training in environmental microbiology. Various projects are carried out with the assistance of the nutritionists and the microbiologist from the dairy and swine sectors.

Health, welfare and society: This group consists of nine researchers: four with expertise in molecular biology and five with expertise in immunology, genetics, lactation biology, ethology, pre-slaughter stress and meat quality.

Production: Seven nutritionists in the dairy sector and two in the swine sector, an endocrinologist, a nutritionist specializing in forage quality, and a sheep production researcher carry out work aimed at increasing the profitability and competitiveness of livestock operations.

Ongoing projects

Environment

- Development of low temperature anaerobic digestion technology permitting biogas capture and utilization, odour reduction and pathogen elimination, as well as nutrient recovery from effluents;
- Development of quantification methods and practices to reduce gaseous emissions (capture of emissions, biofiltration, manure pit and effluent management, and building design);

- Development of membrane filtration technology to treat pig slurry with a view to producing nitrogen fertilizer. This technology can be adapted to different types of effluent (wastewater, greenhouse effluents and whey) and different production volumes;
- Development of strategies for using secondary metabolites to adjust microbial fermentation in the rumen, improve feed efficiency and reduce the environmental impacts of dairy production.

Health, welfare and society

- Identification of metabolic techniques for improving lactation persistency in dairy cows, increasing their longevity and promoting their health;
- Measurement of the effects of biological molecules on disease resistance and immune functions;
- Identification of immunological and genetic factors associated with reproduction, production and health;
- Evaluation of the effects that probiotics have on the ruminal and intestinal microflora of piglets and on their immune responses;
- Evaluation of the impacts of genetics on gene expression and production of biologically active molecules;
- Development of indicators for assessing animal welfare based on type of housing, husbandry practices and human-animal relationships;
- Development of protocols for swine transport and for handling before and during loading, during unloading and during slaughter, in order to improve animal welfare and meat quality.

Production Type

Dairy

- Study of protein metabolism in cows, particularly the conversion of dietary protein to milk protein, and quantification of nitrogen transit in the digestive system and throughout the body;
- Evaluation of different metabolic pathways of lipids, antioxidants and B-complex vitamins with the aim of improving dairy cow metabolic efficiency, reproduction and health and producing milk enriched with these bioactive components;
- Assessment of the effects of supplementary B-vitamins on protein and energy metabolism in dairy cows;
- Development of strategies to improve dairy cows' utilization of nitrogen from forage plants by increasing the carbohydrate concentration of the plants;
- Development of new methods to control bovine mastitis.

Swine

- Evaluation of the effects of supplementation with B-complex vitamins and trace minerals on sow growth and reproduction;
- Development of new dietary strategies to stimulate mammary gland growth in gilts and sows;
- Development of precision feeding technology for growing pigs and gilts;
- Identification of genes that are closely associated with pig reproduction and production traits.

Beef cattle

- Development of strategies to increase concentrations of CLA and omega-3 fatty acids in beef;
- Development of strategies to increase the contribution of grazing to the beef cattle diet.

Collaboration with other organizations

Our research teams participate in and develop many collaborative scientific research initiatives at the national (other departments and universities) and international levels. The researchers also work with producer associations (swine, dairy, beef, sheep, flax and canola) and with a variety of industry stakeholders, such as animal feed companies, breeders' associations, engineering firms and environmental technology firms.

Looking to the future

Strengths

Thanks to the combined expertise of its staff and its unique facilities for environmental and livestock production research, the Centre has the capacity to address a wide range of environmental and production issues, while promoting animal health and welfare. We are working to develop animal production systems that are beneficial to animal health and welfare and are environmentally sustainable, by:

a) Minimizing greenhouse gas emissions and ammonia emissions and reducing odours generated by livestock production activities with a view to improving air quality;

b) Adding value to effluents generated by livestock operations and agri-food businesses through various approaches such as the production of green energy;

c) Removing biological contaminants, such as pathogens, prions, antibiotics and hormonal residues, from farm effluents;

d) Protecting sensitive ecosystems and biodiversity;

e) Improving animal health while reducing disease incidence through enhanced immunological resistance and developing disease prevention strategies;

f) Optimizing production systems that are suited to animal needs;

g) Developing value-added products of animal origin in response to market requirements;

h) Designing management practices that allow fortification of products of animal origin with components that are beneficial to human health;

i) Improving the organoleptic properties of products of animal origin;

j) Minimizing the presence of undesirable components in products of animal origin;

k) Increasing our understanding of the factors favouring social acceptance of new technologies, practices and production methods;

l) Increasing the profitability of production through the better use of resources, management and genetic potential.

Infrastructure

Environment

Our environmental research facilities are unique in Canada. They house a number of technologies (bioreactors, biofilters, membrane filtration, ozone generator, ultraviolet [UV]) for treating slurry, manure and other agri-food industry residues and effluents. These technologies can treat a wide range of volumes, from a few litres to several tonnes. The facilities allow the full development of technologies and best management practices, from feasibility studies to the evaluation of scale-up with large volumes.

They also allow research involving potentially harmful substances (methane, ammonia, pathogens) to be carried out meticulously and in complete safety. The Centre has an olfactometer that is used for the objective quantification of odours. In general, the facilities enable the staff to conduct research with the aim of minimizing the environmental impact of livestock production on dairy and swine farms.

Livestock facilities

The Centre has a new dairy complex, which opened in October 2010, and has 140 cow stalls, a temperature-controlled metabolic room (32 stalls), two controlled environment chambers, in addition to a surgery room for cows and swine.

In other buildings, we have biosafety level 2 facilities for projects on dairy cows and, in the near future, the facilities are slated to be adapted to permit projects focusing on swine.

The Centre has a modern swine complex that houses a permanent herd of 100 sows, as well as facilities for conducting metabolic studies, feed studies and behavioural observations. An experimental slaughter plant is available to researchers for projects aimed at reducing pre-slaughter stress and improving pork quality. There are also laboratories for assessing meat quality.

There is also a facility for research on beef cattle in Kapuskasing, Ontario that can house 140 animals.

Technology platforms

The research teams have access to a wide range of equipment for conducting research in fields related to cellular biology and immunology (animal cell culture cabinets, cytometry), genomics and molecular biology (quantitative PCR, DNA sequencer, genotyping, microarray scanner, etc.) and for physico-chemical analyses (nutrient analyzer, gas chromatography, etc.).

SECTION 3 Organizing Committee

Chairperson's Opening Remarks

The DSRDC is proud to launch its first annual animal science symposium. This type of event is welcomed by senior scientists and draws an enthusiastic response from research teams. The primary motivation of the organizers was to create an annual event that would provide the opportunity for multidisciplinary research teams to share information about their scientific endeavours. Many challenges were involved in bringing together research teams working in a variety of fields such as nutrition, forage quality, meat quality, agricultural engineering, microbial ecology, functional genomics, immunology, endocrinology, metabolism, and animal behaviour and welfare. In the age of communications, networking, partnerships and strategic clustering, what could be more effective than staging an event to bring together dynamic teams with complementary expertise. The DSRDC's annual animal science symposium is therefore a strategic gathering that supports collaborative efforts devoted to innovative science.

This event could not have taken place without the exceptional dedication of the organizing committee. I therefore thank all the committee members for helping to bring the idea to fruition. Thanks and enjoy the Symposium!

Nathalie Bissonnette

ORGANIZING COMMITTEE

Guylaine Talbot, Ph.D., Research Scientist
Hélène Lapierre, Ph.D., Research Scientist
Martin Lessard, Ph.D., Research Scientist
Josée Toulouse, Head of the Documentation Centre
Hélène Lavigne, Research Secretary
Pauline Bilodeau, Agr., M.Sc., Technology Transfer Officer
Nathalie Bissonnette, Ph.D., Research Scientist

SECTION 4 List of Conferences

Pierre Lacasse – Lactation biology

CONTROLLING THE IODINE CONCENTRATION IN MILK

S. I. Borucki Castro,* R. Berthiaume,* A. Robichaud,† and *P. Lacasse
* DSRDC; Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada J1M 0C8
† Health Canada, Food Directorate, Longueuil, QC

At Health Canada's request, a research program was implemented with a view to identifying the key factors determining the iodine content in milk. In an initial Canada-wide survey undertaken to identify the factors influencing milk iodine content, it was determined that the iodine content of feeds and the use of iodine-based teat dips contribute to the iodine levels in milk. A second survey found that cow feed is naturally low in iodine and that most of the iodine in their feed is deliberately added to the ration. An initial experiment showed that there is a linear relationship between the iodine concentration in the cow diet and the iodine concentration in milk. This study not only revealed that the iodine in teat dips contributes part of the iodine present in cow's milk, but also that the way teat dips are used has a significant impact on milk iodine levels. Finally, a second experimental study showed that when a suitable iodine-based teat dip sanitizer is applied prior to milking and wiped off completely, there is little effect on the milk iodine content. However, the use of a post-dip sanitizer before milking or incomplete wiping has a major impact on the milk iodine level. These results have been used to develop a best practices guide, and there is every indication that the problem is being resolved.

Élisabeth Carbonneau – Lactation biology

REDUCING METABOLIC STRESS OF DAIRY COWS DURING THE TRANSITION PERIOD BY PARTIAL MILKING OR NURSING

Élisabeth Carbonneau[a], Anne Marie De Passillé[c], Jeff Rushen[c] Brian Talbot[a] and Pierre Lacasse[b]
[a] Université de Sherbrooke, QC, Canada,
[b] AAFC-Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada
[c] Pacific Agri-Food Research Centre, Agassiz, BC, Canada

During the transition from pregnancy to lactation, the sudden increase in nutrient demand for milk production causes metabolic perturbations and high incidences of metabolic diseases in high yielding cows. We previously showed that limiting milk yield by milking once a day during the first wk of lactation improved metabolic status but reduced milk production during the following weeks (JDS 92:1900). In this study, we examined if limiting milk harvest postpartum while maintaining milking stimulus could improve the metabolic status of cows without reducing overall milk production. 47 Holsteins cows were allocated to three treatments, balanced for parity and milk production: 1) cows were milked completely twice a day from calving (control); 2) cows were partially milked twice a day until d5 after calving (partial); 3) cows were left with the calf to suckle from the dam until d5 and were milked once a day from d3 to d5 (nursing). All cows were milked twice a day from d6 to the end of the experiment (d63). During the treatment period (d1 to d5), milk production averaged 27.3 and 9.7 kg/d for control and partial treatments, respectively. There was no residual effect (P=0.7) of treatments on milk production which averaged 47.5, 45.9 and 46.4 kg/d for the control, partial and, nursing treatments, respectively, between wk2 and 9. The DMI of the cows were similar during and after treatment (P>0.2). From wk2 to 9, milk protein and lactose content were not affected by treatments, but milk fat content tended (P=0.06) to be higher in control cows than in cows where milk harvest was limited (partial + nursing). Blood concentrations of glucose (P<0.001) and phosphorus (P<0.05) were lower and the concentrations of NEFA (P<0.05) and BHBA (P<0.0001) were higher in control cows than in the other cows during the treatment period. The positive effects on glucose and BHBA remained significant (P<0.05) up to d28. There was no effect of treatments on blood urea, calcium and haptoglobin. These results suggest that reducing milk harvest postpartum while maintaining milking stimuli reduces metabolic stress without compromising productivity of high yielding dairy cows.

FUNCTIONAL GENOMICS FOR DISEASE RESISTANCE IN DAIRY COWS

Nathalie Bissonnette
Dairy and Swine Research and Development Centre
Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada J1M 0C8

In dairy production, although general models exist for predicting cow performance, there are no models for predicting disease resistance. This relates to the fact that the environment and nutrition modulate and alter the genome without modifying its sequence, thereby conditioning the animal's physiology. A genomics approach is based on genetics (nucleotide sequence) but also considers the dynamic relationship between the animal's genetic predisposition and its environment (gestation, nutrition, stress, etc.). The next major challenge in animal health will be to integrate nutrigenomics, epigenetics and immunogenetics in research in order to further develop fundamental and contextual knowledge.

Dr Bissonnette's team is working on a multi-faceted molecule called osteopontin (OPN). Initially identified as a structural molecule, osteopontin was later found to be involved in T-lymphocyte activation. Recent studies have also linked OPN to a variety of autoimmune diseases, cancers and other pathological situations, in which resistance to intracellular pathogens is implicated. Now recognized as a cytokine, osteopontin plays numerous roles, notably in adhesion, scarring, apoptosis, and innate and acquired immunity. Our team has observed that OPN is a gene that is rapidly induced in response to a mammary gland infection in cows. Through genetic analysis, we have identified allelic variants associated with estimated breeding value for somatic cell score, which is an indicator of mammary health. Functional analysis of the genetic variants revealed that some DNA polymorphisms influence gene expression, which can be investigated by looking at the link between the amount of OPN secreted in milk and the animal's genotype. Studies on this molecule are ongoing. In addition, Johne's disease, an incurable disease of cattle, is caused by an intracellular pathogen; OPN overexpression has been identified at the sites of Johne's disease lesions. Since certain substances such as vitamin D and butyrate can influence OPN expression, research into the activity of this pleiotropic molecule which is implicated in various aspects of animal health should address nutrition as well as genetic predisposition.

TRANSCRIPTIONAL FACTORS SP1 AND SP3 INFLUENCE DIFFERENTIALLY THE REGULATING SEQUENCE OF THE BOVINE OSTEOPONTIN GENE

N. Bissonnette and C. Thibault
Dairy and Swine Research and Development Centre
Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada J1M 0C8

Osteopontin is a pro-inflammatory molecule which has been involved in numerous physiological aspects, from wound healing to metastasis. In cattle, osteopontin was associated to the paratuberculosis disease. In a previous study, we have identified DNA polymorphisms (SNP) in the osteopontin gene (*SPP1*) associated with the mammary health status of lactating cows. In order to better understand the factors that govern the expression of this gene, the activity of its regulating sequence (i.e. promoter) was study in vitro.

The most prevalent haplotypes (H1–H3) of the *SPP1* promoter were cloned. Two SNP are located in the 5' untranslated region and the third SNP is present in the first intron. The haplotype promoter sequences were analyzed in silico for identification of transcription factor recognition sites using the TRANFAC software. The luciferase reporter constructs of the haplotype containing the 1736-bp regulating sequence were compared in co-transfection assays with, without, or in presence of both SP1 and SP3 using the BOMAC (bovine macrophage), MAC-T (bovine mammary epithelial) and the MCF7 (human mammary epithelial) cell lines.

The basal activity of H2 was lower than H1 and H3 (P = <.0001) in BOMAC and MCF7 cells. In MAC-T, the difference remained significant for H2 compared to H1 (P = 0.031) but with a tendency for the allele H3 (P = 0.066). In presence of the transfection factor SP1, the expression increased globally by ~2-fold in all cell types. In contrast, the transcription factor SP3 had a negative impact on the promoter activity. The co-expression of SP1/SP3 recovered partially the promoter activity. In this study, we demonstrated that the transcription factors SP1 and SP3 impact gene activity and interfere with osteopontin expression.

OSTEOPONTIN SECRETION IN MILK IS LINKED TO THE PRESENCE OF DNA POLYMORPHISMS IN THE GENE (*SPP1*)

N. Bissonnette[1] and P-L. Dudemaine[2]
1 Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, Lennoxville, Quebec.
Sherbrooke, QC, J1M 0C8 Canada
2 Université de Sherbrooke, Sherbrooke, Canada

Osteopontin (OPN) is a phosphoglycoprotein that is present in various tissues and secreted into body fluids, such as bovine milk. It is recognized as an important pro-inflammatory cytokine which has numerous functions, including its role in early T-cell activation during bacterial infection. In a previous study, we found elevated OPN expression in response to mammary gland infection before the first clinical signs of mastitis were observed. Furthermore, several polymorphisms were detected in the 5' untranslated region of the gene (promoter), and the haplotypes associated with estimated breeding value (EBV) for somatic cell score (SCC) were identified. Therefore, in addition to promoting the development of the calf immune system, OPN appears to be linked to mammary gland health. In dairy cows, the protein is secreted during lactation. In the present study, we sought to determine whether EBV is related to the level of secretion of OPN or the presence of isoforms in the milk. We used an antibody panel to characterize OPN isoforms by immunoblotting and to measure the amount of OPN secreted in milk. This enabled us to select the most suitable antibodies to establish an ELISA test. The ELISA test that we developed is highly sensitive (picograms). Variations in OPN concentration, including the level in colostrum, were detected during lactation. The elevated concentration found in colostrum (~500 mg/L) drops significantly at the start of lactation (~100 mg/L milk). The levels detected in cows with haplotypes associated with a low EBV for SCS had higher OPN levels in their milk ($P < 0.0006$). An association therefore exists between the OPN concentration secreted in milk and the cow's haplotype. Eventually, animals with a haplotype corresponding to a high OPN level (or low SCS) could be selected for breeding in order to increase disease resistance in their progeny.

FUNCTIONAL FOODS AND INTESTINAL HEALTH

Martin Lessard
DSRDC, Sherbrooke, Quebec J1M 1Z3

At weaning, the piglet immune system is not fully mature; hence, changes in the animals' environment and diet can have a major impact on their health and intestinal flora. Feeds enriched with probiotics, prebiotics or other functional ingredients may be used to meet the specific requirements of piglets during the weaning period. These dietary supplements have the potential to modulate the intestinal microbiota, inhibit the growth of pathogens, influence the barrier functions of the intestinal mucosa and modulate the immune response. However, the effects of the different supplements on intestinal flora composition and the intestinal immune response have not been fully elucidated. A better understanding of these effects and the underlying mechanisms would make it possible to develop feeds that are better suited to piglet needs and will have a positive impact on their health after weaning. The objectives of our research program are to assess the potential of probiotics and functional foods (milk by-products, bovine serocolostrum, cranberry, essential oils, oligosaccharides) to influence intestinal flora composition and modulate intestinal physiological properties and immune function in weaned piglets.

The results obtained to date show that the probiotic *Pediococcus acidilactici* can influence the microbiota in the ileum and modulate the inflammatory response of weaned piglets infected with enterotoxogenic *Escherichia coli*. Other in vitro studies, in which the intestinal IPEC J2 and Caco-2/15 epithelial cell lines were used, showed that serocolostrum has the potential to modulate the inflammatory response by affecting the expression and production of various chemokines and cytokines. Further studies are being carried out to gain insight into the mechanisms of action and to develop feeding strategies to enhance the intestinal health of pigs after weaning and reduce the use of antiobiotics in feed.

Jean-Philippe Brousseau - Immunology

EFFECT OF THE PROBIOTICS *PEDIOCOCCUS ACIDILACTICI* AND *SACCHAROMYCES CEREVISIAE BOULARDII* ON THE ILEAL MICROBIOTA OF WEANED PIGLETS

Jean-Philippe Brousseau[1, 2], Frédéric Beaudoin[1], Karoline Lauzon[1], Guylaine Talbot[1], Denis Roy[2] and Martin Lessard[1]
[1] DSRDC, 2000 College Street, Sherbrooke, Quebec J1M 1Z3
[2] INAF, Pavillon des Services, Université Laval, Quebec, Canada G1V 0A6

Major changes in the intestinal microbiota of pigs are associated with weaning. In Canada, antibiotics are added to feeds to minimize the adverse effects of weaning. This use of antibiotics has been called into question because of the risk of development of antibiotic resistance in micro-organisms. This is the main reason the European Union instituted a ban on the use of antibiotics in feed to promote growth. It is therefore crucial to develop alternatives to the use of antibiotics in livestock feed. Although probiotics hold promise in that regard, efforts need to be devoted to characterizing their modes of action.

Two piglets per litter from 40 litters were used in a study aimed at evaluating the influence of the probiotics *Pediococcus acidilactici* (PA) and *Saccharomyces cerevisiae boulardii* (SCB) on the ileal microbiota. The animals were divided into 5 treatment groups: 1) PA, 2) SCB, 3) PA+SCB, 4) antibiotics in feed (ATB) and 5) no probiotics or antibiotics (CTRL). Beginning 24 hours after birth, each piglet received a daily probiotic dose of 1 x 109 CFU. After 37 days of treatment, the piglets were euthanized in order to collect the contents of the ileum. The Terminal Restriction Fragment Length Polymorphism (T-RFLP) approach was used to identify the bacterial populations comprising the microbiota.

From the T-RFLP profiles obtained, two diversity indices—the Shannon Index and the Evenness index—were used to assess microbial diversity. A decrease in diversity was found in the ATB and PA groups compared with the CTRL ($P < 0.05$). The relative percentage of the different phyla present was determined, and the diversity of bacteria in the phylum Firmicutes was found to be higher ($P < 0.05$) in ATB, PA and SCB compared with the CTRL group, whereas the diversity of Actinobacteria was lower ($P < 0.05$) in PA compared with the CTRL. A major terminal restriction fragment (177 bp), associated with the genus *Lactobacillus*, was more abundant ($P < 0.05$) in the pigs in the ATB and PA groups compared with the CTRL.

In conclusion, the results show that the administration of PA and antibiotics has a similar effect on the ileal microbiota of weaned piglets, as well as on specific microbial populations.

Mylène Blais - Immunology

EFFECT OF SEROCOLOSTRUM ON INFLAMMATORY RESPONSE GENE EXPRESSION IN PORCINE INTESTINAL EPITHELIAL CELLS (IPEC J2) AND HUMAN COLON CANCER CELLS (CACO-2/15)

Blais M.1, Chambers J.1, Beaudoin F.1, Gauthier S.2, Pouliot Y.2 and Lessard M.1
[1] DSRDC, Sherbrooke, QC, Canada
[2] Département des sciences des aliments et de nutrition, Université Laval, QC, Canada

A number of beneficial effects are associated with the consumption of colostrum after birth, including maturation of the intestinal epithelium and development of the immune system. Most of these beneficial effects appear to be mediated by bioactive peptides present in the serocolostrum, such as growth factors and antimicrobial and immunoregulatory peptides. The goal of this study was to determine the effect of serocolostrum on inflammatory response gene expression in the porcine intestinal epithelial cell line IPEC J2 and in the human colon cancer cell line Caco-2/15. METHODOLOGY. IPEC J2 cells were stimulated with bacterial molecules (*Salmonella typhimurium* and *Escherichia coli*, heat-killed and sonicated), in the presence or absence of serocolostrum. The activity of the transcription factor NF-kB was assessed through transient transfection and luciferase activity assays. In addition, the transcriptional activity of the promoters IL-8 and IL-6 was measured in Caco-2/15 cells using the reporter genes IL-8luc and IL-6luc. The expression of different inflammatory response genes was assessed in IPEC J2 cells by real-time PCR. RESULTS. Serocolostrum impaired the transcriptional activity induced by bacterial molecules, as measured using the NF-kB, IL-8, and IL-6 constructs in luciferase assays. Furthermore, serocolostrum suppresses induction of the expression of IL-8 and IL-6 in porcine intestinal IPEC J2 epithelial cells. CONCLUSION. Serocolostrum exhibits anti-inflammatory effects in IPEC J2 and Caco-2/15 cells.

Candido Pomar – Precision feeding

PRECISION FEEDING CAN SIGNIFICANTLY REDUCE FEEDING COST AND NUTRIENT EXCRETION IN GROWING ANIMALS

C. Pomar[1], L. Hauschild[1,2] G.H. Zhang[1,3], J. Pomar[4] and P.A. Lovatto[2]
[1] Agriculture and Agri-Food Canada, Sherbrooke, QC, J1M 1Z3, Canada
[2] Universidade Federal de Santa Maria, Santa Maria, RS 97119-900 Brazil
[3] Northwest A&F University, Yangling, Shaanxi Province 712100, P. R. China; 4Universidat de Lleida, 25198 Lleida, Spain

Precision feeding is an agricultural concept that relies on the existence of between-animal variability and involves the use of feeding techniques that allow the right amount of feed with the right composition to be provided at the right time to each pig in the herd. Precision feeding is proposed as an essential approach to improve nutrients' utilization and thus reduce feed costs and nutrient excretion. Precision feeding requires accurate knowledge of the nutritional values of feed ingredients and nutrient requirements as well as tools to accurately formulate and deliver daily tailored rations to individual animals.

Information of individual pigs body composition, feed intake and growth performance was used to estimate individual nutrient requirement and population responses to varying levels of dietary nutrients. A simulation study was then undertaken in which the pigs were fed either according to a typical three-phase feeding program (common for all pigs within the population) or a daily tailored feed to each pig as could be provided using precision feeding techniques. These feeding strategies were used to evaluate the consequence on feed cost and on N and P excretion. Feeding cost was determined according to common commercial pig feeds sold in Quebec, Canada. The impact of reducing P intake was not incorporated into the feed cost calculations because of the lack of information. As expected, simulated N and P retention was not affected by the feeding method. However, feeding pigs with daily tailored diets reduced N and P intake by 25% and 29%, respectively and nutrient excretions were reduced both by more than 38%. Feed cost was 4.7% lower for pigs fed daily tailored diets. Some pigs received however more N and P when fed the daily tailored diets than when fed in the three-phase feeding program. In fact, population protein and P requirements were established in this study to optimize average daily gain of the population. These population estimates were 12% higher than the requirement of the average pig and corresponded to the requirement of pigs in the 82% percentile of the population. Estimated nutrient requirements did not include safety margins as normally used in commercial feeds and therefore, the simulated N and P reductions are probably underestimated. Phosphorous excretion should however be interpreted with caution because actual models simulating P retention seldom take into account the effect of P intake in P retention and bone mineralization. Precision feeding can therefore be an efficient approach to significantly reduce feeding costs and the excretion of N and P in growing animals' production systems.

EFFECT OF PHOSPHORUS AND CALCIUM DEPLETION-REPLETION PROTOCOLS ON THE DIGESTIVE AND METABOLIC UTILIZATION OF PHOSPHORUS AND CALCIUM IN GROWING PIGS

M.P. Létourneau-Montminy*[1], P.A. Lovatto[2] and C. Pomar[1]
*[1] Agriculture and Agri-Food Canada, Sherbrooke, QC, J1M 1Z3, Canada
[2] Universidade Federal de Santa Maria, Santa Maria, RS 97119-900 Brazil

Reducing the amount of phosphorus (P) supplied in the swine diet and optimizing phosphorus utilization are effective ways to increase the sustainability of swine production. The effect of dietary calcium (Ca) and P restrictions (i.e. depletion) and recovery phases (i.e. repletion) on the digestive and metabolic utilization efficiency of P and Ca was investigated in growing pigs. Seventy-two pigs were fed a three-phase feeding program (25–50, 50–80 and 80–110 kg live weight) with either a control feed (C), which provided the recommended amounts of nutrients or a diet with 40% less P and Ca (L); both feeding programs had a constant Ca: digestible P ratio. Six dietary protocols were tested: CCC, CCL, CLL, LCC, LLC and LLL. The bone mineral content (BMC, g) of the lumbar region was measured by dual-energy X-ray absorptiometry (DXA) at the beginning and end of each of the growth phases. Total fecal and urine collection was carried out during growth phases two and three. BMC was lower at the end of the first growth period in pigs that received feed L compared with feed C (29%, $P < 0.001$). At the end of the second growth period, the bone mineral deposition rate (g/d) was higher in these animals compared with the controls (CC and CL, 23%, $P < 0.001$). During the third period, the pigs that received diet LLC absorbed 26% more Ca ($P < 0.01$) and had 53% higher bone mineral deposition ($P < 0.05$) than the pigs fed diet CCC. Digestive and metabolic adaptations allowed pigs that were fed diet C during the last or last two growth periods, that is, after 2 (LLC) depletion periods or 1 (LCC) depletion period, to reach a bone mineralization level similar to that of the controls (CCC). Although P excretion was decreased by 18% in LLC pigs relative to CCC pigs, before practical applications can be developed, further study is required in order to understand more fully the degree, the timing (physiological stage) and the duration of the depletion and repletion periods. Nonetheless, the metabolic adaptations identified in this study point the way to a promising approach to improving P utilization efficiency in pigs.

IMPACT OF STRESS DURING GESTATION AND ENRICHMENT DURING LACTATION ON THE MATERNAL BEHAVIOUR OF SOWS

Nadine Ringgenberg[1,2], Renée Bergeron[3], Nicolas Devillers[2]
[1] University of Guelph, Guelph, ON, Canada
[2] Agriculture and Agri-Food Canada, Dairy and Swine R&D Centre, Sherbrooke, QC, Canada
[3] University of Guelph, Alfred Campus, Alfred, ON, Canada

The impact of a social stress in gestation and an enriched pen in lactation on sow maternal behaviour was studied in a 2 × 2 factorial experiment. At breeding, 41 Yorkshire-Landrace sows were assigned to either a social stress treatment (T) during mid-gestation or a control group (C). During lactation, half of the T and C sows were housed in straw enriched pens (E) (1.57 m × 4.10 m) and the others in standard farrowing crates (S) (0.68 m × 2.10 m). The social stress consisted in mixing each T sow with two unfamiliar, larger and older sows twice for one week, from d 39-45 and 59-65 of gestation. Aggressive behaviour was observed and scratch and lesion scores were recorded to confirm that a social stress occurred. During lactation, the responses of sows to piglet vocalization playbacks were observed on d 3 and 21. In addition, postural budgets of sows were automatically detected using accelerometers taking measurements every 5 s on d 5 and 19 for 24 h. Sow-initiated social contacts with the piglets were observed continuously from video recordings on d 6 and 20 of lactation. On d 21, stress during gestation had an impact on the response of sows to isolated piglet vocalisation playbacks with T sows grunting later than C sows (16.25 ± 2.06 s vs. 12.95 ± 1.27 s, P = 0.035). In early lactation, T sows spent more time lying ventrally than C sows (11.08 ± 1.41 % vs. 7.13 ± 1.35 % of time, P = 0.0072). Furthermore, the stress in gestation had an impact on the use of environmental enrichment, with T sows tending to spend less time in the nesting straw area of the pen than C sows (38.57 ± 8.36 % vs. 58.37 ± 9.53 % time, P = 0.061). Housing conditions also impacted maternal behaviour with E sows tending to have more social contacts with their piglets than S sows in early lactation (28.44 ± 8.69 vs. 19.95 ± 8.90 contacts per , P = 0.058), and tending to spend more time lying ventrally than S sows (14.60 ± 1.38 vs. 20.93 ± 4.25 % time, P = 0.067). In conclusion, a social stress during mid-gestation negatively impacted the maternal behaviour of sows. While an enriched farrowing pen allowed for more opportunities to express maternal behaviours, it did not counteract the negative effects of gestation stress.

Hélène Lapierre – Physiology, endocrinology

A LARGE SUPPLY OF PHENYLALANINE IS NOT OXIDIZED BY THE MAMMARY GLAND OF DAIRY COWS

S. Lemosquet[1], G.E. Lobley[2], R. Koopman[3], L.J.C. van Loon[3], A.K. Kies[4] and H. Lapierre[5]
[1] INRA UMR1080 Dairy Production, Saint-Gilles, France
[2] Rowett Institute of Nutrition and Health, University of Aberdeen, UK
[3] Department of Human Movement Sciences, NUTRIM, Maastricht University, The Netherlands
[4] DSM Food Specialities, R&D, Biochemistry and Nutrition Department, Delft, The Netherlands
[5] Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada

In the mammary gland (MG) of ruminants, Group 1 amino acids are reported to be quantitatively transferred into milk protein, on a net basis (Mepham, 1982. JDS 65:287). Phenylalanine (Phe) and its first metabolite, tyrosine (Tyr), are included in this group (as Phe+Tyr). Oxidation of Phe+Tyr across the MG would preclude estimation of mammary plasma flow by the Fick principle: this calculation assumes that net uptake of these AA is quantitatively transferred into milk protein. To determine if Phe+Tyr are oxidized across the MG, two Holstein dairy cows (23.3 kg/d DMI; 49.9 kg/d of milk at 27.7 g/kg of true protein content) received a diet based on maize silage (VanLoon *et al*., 2009. JDS 92:4812). They received a large jugular infusion of L[1-^{13}C]Phe (100 g/d) over 2 d while the diet was estimated to supply 125 g/d of intestinal Phe. The cows were equipped with catheters in the tail artery and in the milk vein of the left udder. Blood samples were collected hourly during 3 periods: before the start of the infusion (n=3) and from 6 to 11 h (n=6) and 30 to 35 h (n=6) of the infusion period. Concentrations of CO_2 and isotopic enrichments (IE) were determined on blood gas analyser and on an isotopic ratio mass spectrometer, respectively. Labelled $^{13}CO_2$ (concentration × IE) did not increase between arterial and mammary venous blood, i.e. the $^{13}CO_2$ arterio-venous difference (AVD) did not differ from zero (P>0.20) and averaged +0.01 and +0.14 ± 0.11 μM for cow 1 and 2, respectively. These values need to be corrected for sequestration of $^{13}CO_2$ across the MG, ranging from 1.2 to 4.8% of arterial $^{13}CO_2$ inflow in three dairy cows (Raggio *et al*., 2006. JDS 89:4340). Based on the extremes of these values, adding this CO_2 sequestration across the MG would result in a $^{13}CO_2$ AVD of between -0.04 to -0.37 μM. Negative values indicate net oxidation but this represents only 0.1 to 0.7 % of labelled Phe+Tyr inflow to the MG. In conclusion, in the current study, even a large and imbalanced supply of Phe did not result in significant oxidation of Phe+Tyr across the MG. This result suggests that under normal feeding conditions, any oxidation of Phe+Tyr is insufficient to preclude the use of these AA to estimate mammary plasma flow using the Fick principle.

Mactar Seck - Physiology, endocrinology

PARTICLE SIZE AND ENDOSPERM TYPE OF DRY GROUND CORN ALTER APPARENT RUMINAL SYNTHESIS OF B-VITAMINS IN LACTATING DAIRY COWS

M. Seck*[1,3], M. S. Allen[2], P. Y. Chouinard[3], C. L. Girard[1]
[1]Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada
[2]Department of Animal Science, Michigan State University, East Lansing, MI, USA,
[3]Departement de sciences animales, Universite Laval, Quebec, Quebec, Canada

Effects of dry ground corn varying in particle size and endosperm type on apparent ruminal synthesis (AS) of thiamin (B1), riboflavin (B2), niacin (B3) and vitamin B6 (B6) were evaluated using eight ruminally and duodenally cannulated dairy cows. The experiment was a duplicated 4×4 Latin square design with a 2×2 factorial arrangement of treatments. Main effects were corn grain vitreousness (floury or vitreous) and particle size (medium or fine). Endosperm was 25% vitreous for floury treatment and 66% vitreous for vitreous treatment. The fraction of grain passing through a 1.18 mm sieve was 43% for medium, vitreous, 42% for medium, floury, 57% for fine, vitreous and 62% for fine, floury. Diets included alfalfa silage, corn treatments, protein supplement, minerals, vitamins, and contained 29.2% starch, 27.2% NDF and 18.3% crude protein. Corn grain treatments supplied 86.2% of the dietary starch. Grinding and endosperm type had no effects on daily intakes of B-vitamins except for B2 where fine grinding decreased daily intake (175 vs. 181 ± 8.6 mg/d, P=0.04). Reducing particle size increased duodenal flow (DF) of B2 (391 vs. 327 ± 26.6 mg/d, P<0.01), B3 (3513 vs. 2939 ± 317.0 mg/d, P=0.01) and tended to increase DF of B1 (50.7 vs. 40.4 ± 3.7 mg/d, P=0.07). Floury treatment increased DF of B3 (3453 vs. 3000 ± 317.0 mg/d, P=0.04). Fine grinding increased AS of B2 (215 vs. 146 ± 23.8 mg/d, P<0.01), B3 (2671 vs. 2083 ± 289.5 mg/d, P<0.01) and B1 (8.4 vs. -1.4 ± 2.7 mg/d, P=0.05) while floury endosperm increased AS of B3 (2602 vs. 2152 ± 289.5 mg/d, P=0.03). DF and AS of B6 were not affected by treatments (P>0.13). B1 AS was correlated negatively with true ruminal digestibility of organic matter expressed as percentage of intake (P<0.01, r=-0.48) or as kg/d (P=0.02, r=-0.40). Duodenal flow of microbial nitrogen was correlated positively with AS of B2 (P<0.01, r=+0.52), B3 (P<0.0001, r=+071) and B6 (P=0.02, r=+0.41). B-vitamin supply to dairy cows is affected by dry corn particle size and to a lesser extent, by endosperm type.

Daniel Ouellet – Nutrition, physiology

EFFECT OF DIETARY NITROGEN LEVELS AND YEAST SUPPLEMENTATION ON APPARENT DIET DIGESTIBILITY AND MICROBIAL POPULATION IN THE RUMEN CONTENT OF DAIRY LACTATING COWS

Daniel Ouellet and Johanne Chiquette
Dairy and Swine Research and Development Centre, AAFC, 2000 College, Sherbrooke, QC J1M 0C8, Canada

Eight rumen-fistulated Holstein dairy cows (679 kg BW; SEM = 5) were used in a duplicated 4 x 4 Latin square design, with a 2 x 2 factorial arrangement of treatments to evaluate the effect of dietary nitrogen levels and yeast supplementation on apparent diet digestibility and rumen content population of dairy lactating cows. Isoenergetic diets, highly or moderately deficient in metabolizable protein [-22% (HD) or -14% (MD) less than requirements], were fed with or without yeast supplement (10 g/head/d of a mixture of *Aspergillus oryzae* and *Saccharomyces cerevisiae*). Total mixed ration (60:40 grass silage:concentrate barley based) was fed 12 times daily. Apparent digestibility of DM, OM, NDF, ADF and N were measured. Ruminal fluid content was sampled to estimate protozoa, cellulolytic and total viable bacterial count. Plasma urea concentration was determined. Apparent digestibility of N (69.5% vs. 65.9%; SEM = 1.6) and urinary N excretion (261g/d vs. 162 g/d; SEM = 6) was higher ($P < 0.01$) in MD than in HD. Apparent digestibility (average %) of DM (72.4; SEM = 1.1), OM (74.4; SEM = 1.1), ADF (52.5; SEM = 2.3), and NDF (57.8; SEM = 2.5) were similar among treatments. Yeast supplementation did not affect apparent digestibility parameters. Compared to MD, HD reduced by 48% plasma urea concentration (10.6 vs. 15.7 mg/dL; $P < 0.02$). Average microbial counts were unaffected ($P > 0.05$) by treatments. Protozoa counts [geometric means (cfu/mL) and 95% confidence interval (CI)] were: 2.3×10^5 (CI= 1.6×10^5 to 3.4×10^5), total viable bacteria: 3.1×10^9 (CI = 2.6×10^9 to 3.7×10^9) and cellulolytic bacteria: 3.9×10^7 (CI = 2.8×10^7 to 5.5×10^7). In conclusion, addition of yeast to diets containing up to -22 % the requirements in metabolizable protein had no effect on the apparent digestibility of the diet, ruminal content parameters and plasma urea.

MICRORNA: MECHANISMS OF GENE REGULATION AND APPLICATION TO COW HEALTH

Eveline M. Ibeagha-Awemu
Agriculture and Agri-Food Canada, Sherbrooke, QC, J1M 0C8, Canada

Micro RNA (miRNA), a class of naturally occurring small noncoding RNAs of about 20 to 24 nucleotides in length has emerge as an important regulator of gene expression at the post-transcriptional or translation level. miRNAs are predicted to control the activity of more than 60% of all protein-coding genes and participate in the regulation of almost every cellular process (e.g. development, differentiation, apoptosis, viral infection and disease) investigated in mammals. Dysregulated expression of miRNAs in various tissues has been associated with a variety of diseases, e.g. cancer. miRNAs have been identified and characterized in different tissues in bovine but the role of miRNAs in cow's response to mastitis pathogens is not known. We evaluated the expression pattern of miRNAs in MAC-T cells (mammary epithelia cell line) challenged with heat inactivated *E. coli* or *S. aureus* bacteria (treatments: 0, 6, 12, 24 or 48 hrs) using an Agilent miRNA microarray containing 672 bovine miRNA targets (Jin W, Ibeagha-Awemu EM, Stothard P, Zhao X, Guan LL. 2011). A total of 198 miRNAs were found to be significantly expressed (value > 0, 75th percentile normalization and log 2 transform). Three miRNAs were up-regulated (>2-fold) at all time points and 6 miRNAs were down-regulated (>2-fold) in at least one of four time points in E. coli samples. Similarly for S. aureus samples, 3 miRNAs were up-regulated at greater than 2-fold and 13 miRNAs were down-regulated (at greater than 2-fold) in at least one of four time points. Also, six down-regulated miRNAs and one up-regulated miRNA showed similar trends in cells challenged with both pathogens. This study shows that miRNAs may play a role in cow's response to mastitis causing pathogens and are potential biomarkers for early detection and management of mastitis. However, the miRNA populations that are expressed in the mammary gland in response to bacterial pathogens are not known as well as their exact roles and the genes they regulate.

.....

Bibliographic note / Note bibliographique

In April of 2011, Dr Ibeagha-Awemu joined AAFC as research scientist (animal genomics) where she is interested in (1) studying the role of nutrients on the genetic & epigenetic regulation of lipogenesis & fatty acid composition of milk and adipose tissues; (2) deciphering the genetic and epigenetic factors modulating bovine mastitis and other diseases; and (3) understanding the role of microRNA gene regulation in modulating lipogenesis, fertility, mucosal immunity and mastitis.

Eveline M. Ibeagha-Awemu started her research career in 2000 as animal genomics at the laboratory of Animal Genetics and Breeding, University of Giessen (Germany) where she obtained a PhD in Genetics. Her PhD Thesis used molecular genetic techniques to evaluate genetic diversities in 27 cattle breeds from Africa, Europe and Asia. Following this, she participated in an European Union sponsored project, Econogene (http://www.econogene.eu/) which combined molecular analysis of biodiversity, socio-economics and geostatistics to address the conservation of sheep and goat genetic resources and rural development in marginal agro-systems in Europe. In early 2005 she joined the Department of Animal Science, McGill University in Canada as a postdoctoral fellow and later as research associate where she continued her research activities in the area of animal genetics. Her research activities at McGill included understanding (1) the role of mammary epithelial cells in mounting an immune response after challenge by bacterial pathogens; (2) the proteins and signalling pathways that are activated during E. coli and S. aureus mastitis; (3) the role of microRNA in modulating E. coli and S. aureus mastitis; (4) the role of selenium in neutrophil immune functions and (5) the relationship between sequence variations of important lipogenic genes and milk fatty acids profiles of Canadian Holstein and Jersey cows.

Guylaine Talbot - Environment

METHANOGENESIS IN DAIRY AND SWINE PRODUCTION

Talbot G.*[1], M. Barret[1], D.I. Massé[1], L. Masse[1], M. Kalmokoff[2] and Ed Topp[3]
*1Dairy and Swine Research and Development Centre, AAFC, 2000 College, Sherbrooke, QC J1M 0C8, Canada
2 Atlantic Food and Horticulture Research Centre, AAFC, Kentville NS, B4N 1J5 Canada
3Southern Crop Protection and Food Research Centre, AAFC, 1391 Sandford Street, London, ON N5V 4T3, Canada

The broad objectives of the environment research group are to reduce gaseous emissions, excessive nutrient inputs and biological contaminants; and to treat and add value to agricultural and agri-food residues through anaerobic digestion and membrane filtration. The presentation focuses on the issue of methane emissions from dairy and swine operations. The steps leading to methanogenesis will be discussed and illustrated with reference to a study on methanogen communities in a compartmentalized bioreactor, simulating the storage of slurry in manure pits. After 6 months of operation, the bioreactor permitted the development of methanogen communities that were adapted to their environment and were more effective at producing methane. The mcrA gene which codes for an enzyme essential for methanogenesis was selected as a target for determining the diversity of the communities and the relative levels of enzymatic activity by means of a new molecular profiling technique (LH-mcrA) combined with clone library analysis. The results show that hydrogenotrophic methanogens related to the (genus *Methanoculleus*) were the main active methanogens.

Maialen Barret - Environment

ACTIVE METHANOGENS IN SWINE MANURE STORAGE TANKS REVEALED BY DNA STABLE ISOTOPE PROBING

Barret M.[1], Neufeld J. D.[2], Gagnon, N.[1], Kalmokoff, M.[3], Topp E.[4], Verasteguy, Y.[2] and Talbot G.[1]
[1] Dairy and Swine Research and Development Centre, AAFC, 2000 College, Sherbrooke, QC J1M 0C8, Canada
[2] Department of Biology, University of Waterloo, Waterloo, ON
[3] Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, Kentville NS, B4N 1J5 Canada
[4] Southern Crop Protection and Food Research Centre, AAFC, 1391 Sandford Street, London, ON N5V 4T3, Canada

Emissions of the greenhouse gases (GHG) methane (CH_4) and nitrous oxide (N_2O) from stored manure account for 17% of Canada's total agricultural GHG emissions. In anaerobic storage systems, microbial communities involved in CH_4 production are not known at genus taxonomic level. Acetate was the most abundant volatile fatty acid in these storage tanks and is the main intermediate metabolite in methanogenesis. From two different stored swine manure samples, DNA stable-isotope probing (DNA-SIP) experiments using ^{13}C-acetate were carried out to identify active methanogens and determine if the direct acetoclastic or the indirect non-acetoclastic oxidative pathways were involved. The results clearly demonstrated that the indirect methanogenesis pathway by the hydrogenotrophic *Methanoculleus* spp. was dominant over the direct methanogenesis pathway by the acetoclastic *Methanosarcina* spp. These results were consistent using both DNA fingerprinting by amplicon-length-heterogeneity PCR targeting the *mcrA* gene and *mcrA* gene clone library analyses. These two methanogenic genera were minor populations of the archaeal community in the original stored swine manures and in fresh manure. This suggests that methanogens that are abundant in pig's intestine are not active in storage tanks.