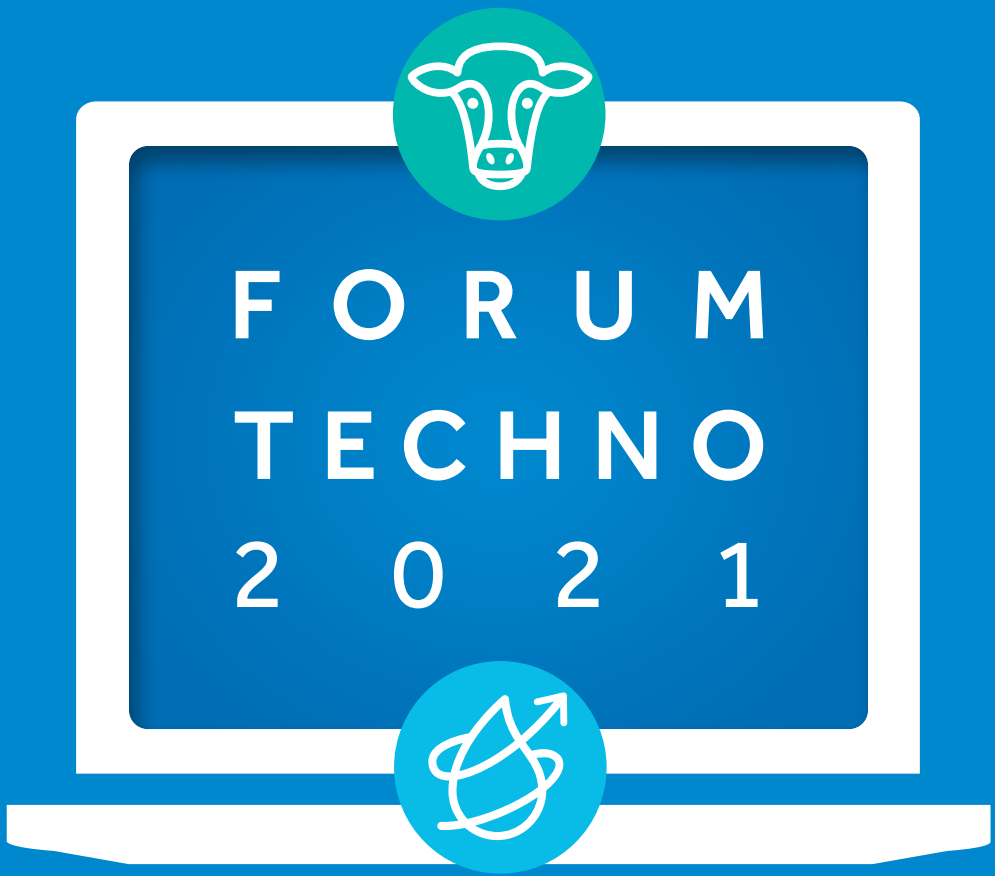


Novalait

Research Catalyst

A Word from the President

Hello everyone,

On behalf of the Novalait Board of Directors, I am pleased to welcome you to the 2021 Forum Techno and to extend a special welcome to our anglophone attendees. This year, we had the opportunity to open up the Forum's activities to participants in the IDF International Cheese Science and Technology Symposium.

In 2020, Novalait celebrated its 25th anniversary. For dairy farmers and processors in Quebec, research is at the core of solutions. Quebec's dairy farmers and processors effectively achieved this vision for innovation by creating an R&D investment company in which they are equal shareholders.

Since 1993, based on annual contributions equivalent to 1.27¢/100 litres of managed milk, Novalait has developed a research portfolio that is now valued at more than $55 million. To this end, Novalait leveraged everything from research partnership programs, tax credits, and revenue-generation through the exploitation of intellectual property.

The purpose of Novalait's research is to bring concrete solutions to dairy farms and plants of all sizes producing a wide range of products. To rise to this challenge, Novalait relies on user committees to establish its research priorities, select, and co-develop its projects with researchers.

Dairy plants also contribute to the research by providing technical advice and samples of all kinds, including milk, dairy products, and co-products—even used separation membranes! For their part, dairy farms open their doors to pilot projects and participate in important data collection processes for research.

Novalait communicates the results of its research through its website, novalait.ca, its partners in the field dedicated to knowledge transfer and, of course, through the Forum Techno—our must-attend event which, this year, is offering solutions to dairy farms and plants through sessions and capsules on the research currently underway.

Novalait earmarks more than 25% of its research budgets for graduate student salaries. Over the past 25 years, more than 300 professionals have received training through our applied research programs. Many of them are still working in the dairy sector. The Forum Techno places great importance on the next generation of professionals and encourages exchanges with students through virtual sessions and the "My Thesis in 180 Seconds" presentations.

Novalait's investments have also attracted and retained bright minds in Quebec to meet the scientific challenges of the dairy industry. We invite you to make the most of the Forum by sharing your challenges related to milk production or dairy product manufacturing with researchers. Novalait's future projects are born out of these conversations. In passing, I would like to note that Novalait is now continuously welcoming project ideas. I invite you to contact our CEO, Ms. Élise Gosselin.

The Forum is a unique opportunity to leverage Novalait's investment in research. Take the opportunity to ask researchers about the applications of their discoveries, share your research interests and, who knows, recruit new talent to innovate and grow!

I wish all of you a successful Forum and a most enriching and fascinating virtual experience!

Michel Couture
President of Novalait

Partners

Platinum

AGROPUR
Dairy Cooperative

DAIRY FARMERS OF CANADA | LES PRODUCTEURS LAITIERS DU CANADA

Gold

Les Producteurs de lait du Québec

LACTALIS CANADA

Saputo

iff

Silver

DAIRY FARMERS of ONTARIO Milk EST. 1965

QUALTECH

Conseil des industriels laitiers du Québec inc.

Canadian Dairy Commission | Commission canadienne du lait

Partners

Bronze

Sectoral

Éditor

Media

Co-organizers

Program

June 8, 2021 - Production-oriented program

8 h 20	**Welcoming Remark**	
	Genetic Section	
8 h 30	**Improving the history of cow health and fertility traits** Claude Robert, Université Laval	
8 h 55	**Pushing back the insemination of cows under metabolic stress** Marc-André Sirard, Université Laval	
9 h 20	**On-going project capsules:** **Vitamins post-calving: a way to increase cow fertility?** Marc-André Sirard	**Comparison of cows resulting from *in vitro* fertilization and artificial insemination** Marc-André Sirard
9 h 30	**Exchange Period**	
	Health and Nutrition Section	
9 h 45	**The benefits of using new, more digestible alfalfa for Quebec's dairy farms** Caroline Halde, Université Laval	
10 h 10	**Using the fatty acid profile of milk to detect and prevent ruminal acidosis in cows** Stéphanie Claveau, Agrinova	
10 h 35	**Exchange Period**	
10 h 50	**Dynamic Break**	
10 h 55	**Three-minutes thesis presentations** **Can subclinical ruminal acidosis be predicted by the fatty acid profile of milk?** Félix Huot **More digestible alfalfa: are they all really?** Marie-Soleil Boucher **Long live the cow: a machine learning approach to analyze the welfare, longevity, profitability, and productivity of dairy cows** Gabriel Dallago	
11 h 05	**Sponsor video: Agropur dairy cooperative**	
11 h 10	**Virtual Student Session - short presentations**	
	Sustainability and Welfare Section	
11 h 40	**Industrial research chair on sustainable life of dairy cattle - Longevity aspect** Elsa Vasseur, McGill University	
12 h 10	**On-going project capsule:** **Sustainable agricultural building design** Sébastien Fournel, Université Laval	
12 h 15	**Exchange Period**	
12 h 20	**Closing Remark**	
12 h 30 - 14 h 00	**"Smart couchs"**	

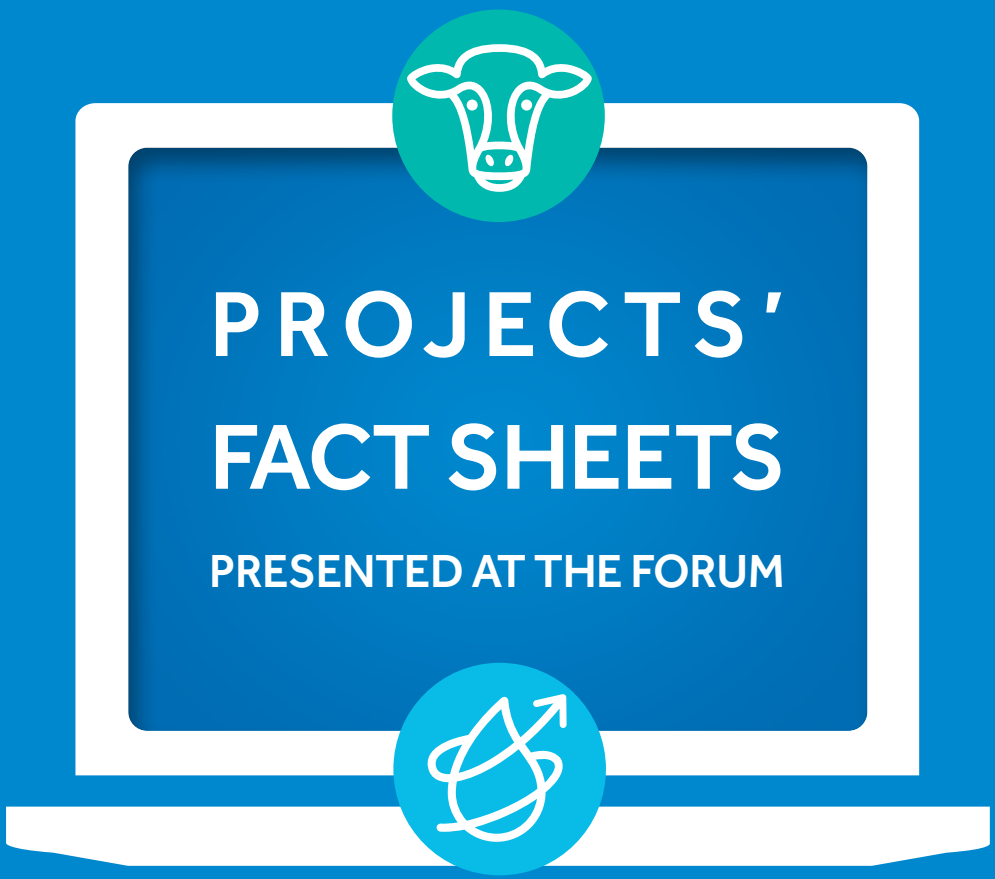
The boxes in green are the "live" sessions

Program

June 10, 2021 - Processing-oriented program

8 h 20	**Welcoming Remark**	
	Microbiology Section	
8 h 30	**History of cheese microflora** Steve Labrie, Université Laval	
8 h 55	**Phages in dairy products** Sylvain Moineau, Université Laval	
9 h 20	**On-going project capsules:** **Microbial biofilms from the farm to the cheese vat** Denis Roy, Université Laval	**Use of recycled bedding and its impact on milk quality** Simon Dufour, Université de Montréal
9 h 30	**Exchange Period**	
	Naturality and Clean label Section	
9 h 45	**Natural food products: better understanding consumer perceptions and behaviour** Joanne Labrecque, HEC Montréal	
10 h 00	**Metabiolac Chair; Harnessing the power of lactic acid bacteria to fight pathogens in the dairy sector** Ismail Fliss, Université Laval	
10 h 15	**Exchange Period**	
10 h 30	**Dynamic Break**	
10 h 35	**Three-minutes thesis presentation** **Antimicrobial systems in cheeses and how to find them?** Rachel Langlois-Deshaies **When buttermilk is under high pressure** Serine Touhami	
10 h 45	**Capsule on the 25th anniversary of Novalait**	
10 h 50	**Virtual Student Session - short presentations**	
	Co-product Valorization Section	
11 h 20	**New approaches to valorize lactose-rich co-products into high value-added products** Salwa Karboune, McGill University	
11 h 35	**An eco-efficient approach to valorizing buttermilk** Guillaume Brisson, Université Laval	
11 h 50	**Increasing eco-efficiency by concentrating dairy fluids** Yves Pouliot, Université Laval	
12 h 05	**On-going project capsules:** **Characterizing the structure of stirred yogurts using digital technology** Sylvie Turgeon, Université Laval	**Novelty in cheese technology** Julien Chamberland, Université Laval
12 h 15	**Exchange Period**	
12 h 30	**Closing Remark**	
12 h 30 - 14 h 00	**"Smart couchs"**	

The boxes in blue are the "live" sessions



PROJECTS' FACT SHEETS

PRESENTED AT THE FORUM

2021

Novalait

Improving the history of health and fertility traits in dairy cows

Duration: 2016-2018

Highlights

- Dairy cattle productivity is influenced by the ways in which the animals are managed and their genetic potential. Through genetic improvement (traditional and genomic), we aim to improve performance by selecting the highest-performing versions of the genes. The traditional approach is to mate the highest-producing animals without taking DNA into account, while genomics aims to select the DNA sequences that are associated with animals exhibiting the highest productivity levels.
- The general model involves having two copies of the best version of the gene, representing the best genetic combination. However, it has been found that certain traits react better to the cross-breeding of different species, most notably traits associated with health and fertility. This means that, for certain traits, the best combination is to have two different versions.
- Dairy production in Quebec and Canada is not based on the cross-breeding of species to promote genetic heterogeneity. Furthermore, it is contraindicated to aim for genome-wide diversification (all of the DNA that is found in each cell) because this would counter the genetic gains made in the last 40 years.
- The project aimed to develop a genomic analysis tool that would leverage the genetic variability present in the Holstein breed to diversify specific regions of the genome that are known to be involved in health and fertility traits.

Objectives

Our hypothesis is that the diversification of specific regions of the genome will help improve health and fertility traits.

The objectives are to develop a genomic analysis tool that will allow us to generate a genetic diversity score, which we can then apply in the assessment of an animal's genetic heritage or to estimate diversification potential through mating. To do so, we must:

1) Identify the regions known to influence the health and fertility of dairy cattle;
2) Choose targets;
3) Develop a genome analysis tool;
4) Program the interface to make it easy to use;
5) Test the tool with genomic data.

Results and potential benefits

Canada has an excellent reputation in regards to the genetic quality of its dairy herds. The driving force of this efficiency in genetic improvement is the result of the amount and precision of performance data collected on farms. Since 2009, genomic selection has complemented traditional genetics. The current model focuses on concentrating the good versions of genes, which decreases genetic diversity. A decrease in the number of bulls also greatly affects the breeds and has led to an uptick in inbreeding rates. It is known that it is more difficult to improve health and fertility traits. This is possibly because these traits are greatly influenced by the environment, in addition to involving a large number of genes that, individually, have very little influence. All dairy genetic selection has turned to genomics and, currently, rising above the rest is difficult because everyone is focusing on the same genetic combination. We believe that we can make a difference in the improvement of health and fertility traits by attempting to diversify regions of the genome that are known to be involved in these traits. By leveraging the species' existing genetic diversity, we will avoid the unfavourable case of interbreeding animals of different species. Given equal genetic values, an animal with a higher genetic diversity score will be better suited for production. In the initial proof of concept, the team genotyped 200 bulls and identified over 900 zones of interest in their genome. They then tested an additional lot of 1,000 dairy cows to validate the regions of the genome related to health and fertility. Their work has demonstrated that it is possible to increase the diversity of the regions of interest by focusing on improving health and fertility traits without neglecting other traits such as milk production. The next step of the project is currently underway to test a larger number of genotyped animals. Ultimately, farmers will be able to use a tool to select bulls in an effort to improve their health and fertility traits through increased genetic diversity.

Innovative aspects

- Non-traditional genetics approach that consists of using the genetic variability present in the Holstein breed to diversify certain regions of the genome responsible for health and fertility characteristics.
- Prototype of customized tool for selecting bulls in order to optimize the health and fertility characteristics of a given cow.

Professional trained

The initial project trained one Master's student, **Alexandra Carrier**, and one research professional, Alexandre Bastien.

A new research professional, **Julien Prunier**, will be involved in the next steps of the project.

For further information

The project's target audience is mainly those who work in the field of genetic selection as well as dairy farmers. The results will be presented at the CRAAQ's Dairy Cattle Symposium (Symposium des bovins laitiers) and Novalait's Forum Techno; additionally, an article has been written for the journal *Le producteur de lait québécois*. A follow-up study is being prepared to validate the project results using a larger sample size.

- Revue *Le producteur de lait québécois*, Décembre 2018, Un outil de sélection personnalisé. Pages 26-27.

Financial contributions

Project 1: Partnership for innovation in dairy production and dairy processing (EPI 2015-2019):

- Fonds de recherche du Québec – Nature et technologies
- Consortium de recherche et innovation en bioprocédés industriels au Québec
- Novalait

Total budget: $186,706

Project 2:

- MAPAQ Innov'Action program
- Lactanet
- Novalait

Total budget: $148,825

Contact persons

Project supervisor:

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Postponing insemination of cows in metabolic stress to Day 120: an idea to validate for the health and profitability of the herds

Duration: 2016-2019

Highlights

- For some cows, the beginning of a new lactation is stressful, and oftentimes even more so in the case of high producers. Their energy metabolism is put under severe strain. Good producers are simply unable to integrate all of the energy necessary for production.
- Energy deficits in the cow, indicated by a high blood BHB level (> 0.8 mmol/L), have an impact on embryo development.
- A specific signature is noted in the embryo, including both transcriptomic, with an immediate effect, and epigenetic, with an effect over the longer term.
- This particular signature causes the embryo to switch to "energy-saving mode", which can reduce its chances of implantation and lead to a different metabolic programming over the longer term.
- Postponing insemination from Day 60 to Day 120 postpartum for cows showing energy deficiency can help avoid this type of embryo programming.
- Prolonging the voluntary waiting period for cows with high BHB levels in their milk (> 0.15 mmol/L) results in a longer interval between calving in these animals.
- Partial budget analysis shows that the impact on net earnings of the herds is marginally positive. Given that there are no economic drawbacks, why not favour a practice that might prove beneficial for the herd in the long term?

Objectives

Thanks to economic and epigenomic analysis, the objective is to provide farmers with the tools needed to determine the energy state of lactating cows, as well as the economic and biological benefits and disadvantages of delaying the insemination of cows with a BHB blood level greater than 1.2mM/L.

Results and potential benefits

Genomic analysis demonstrates that on Day 60, the embryos are different in cows showing high BHB levels. Differences observed at the transcriptomic level indicate an adaptation that consists of switching to an "energy-saving mode", which in the short term could compromise the chances of implantation. In terms of epigenetic programming, i.e. over a longer timeframe, DNA modifications target genes that are particularly important for metabolism, which might suggest an adaptation to an energy-poor environment. Eight heifers from cows with high or low BHB levels were produced from these embryos and epigenetic analysis of their blood indicates approximately 2,000 differences. These indicators must now be sorted and validated before they can be used to monitor the herd and choose the heifers to be retained. These tools are essential for the development of factual management practices based on measurements taken at birth (biomarkers) and in milk (BHB) and quickly adopted by producers.

For the economic portion of the project, a partial budget was created to assess the impact of prolonging the voluntary waiting period for cows with a high BHB level in their milk at the time of the first control. Analyses conducted based on Lactanet's database and scientific publications served as a basis for an assessment adapted to the Quebec reality for herds with average annual productions of < 9,000, between 9,000 and 11,000, and > 11,000 kg of milk. Results show that extending the voluntary waiting period for cows with high BHB levels in their milk only has a marginally positive impact on a dairy farm's net earnings, regardless of its average production (< 9,000 kg of milk: $10.10/cow/year; 9,000 to 11,000 kg of milk: $17.30/cow/year; > 11,000 kg of milk: $8.60/cow/year). In this context, and in light of the potential benefit for the embryos, it would be advisable to adjust our practices.

Innovative aspects

- Discovery of a specific embryo signature, including both transcriptomic (short-term) and epigenetic (longer-term) components.
- Prolonging the voluntary waiting period between calving and subsequent insemination for cows experiencing metabolic stress could help avoid unfavourable embryo programming without having an impact on the company's net earnings.

Professionals trained

This project resulted in the training of one Master's student in genomics, **Catherine Chaput**, and one Master's student in technical and economic management for the dairy industry, **Catherine Couture**. Both students are enrolled at Université Laval's Department of Animal Sciences.

For further information

The results of the project have been transferred by an extension paper, posters session at the Quebec Dairy Cattle Symposium, the Novalait Forum Techno and at some local knowledge transfer day. Already published:

- Revue *Le producteur de lait québécois*, Janvier-février 2020, Repousser l'insémination chez les vaches en déficit énergétique; une idée validée. Pages 30-32.

Financial contributions

Partnership for innovation in dairy production and dairy processing (EPI 2015-2019):

- Fonds de recherche du Québec – Nature et technologies
- Consortium de recherche et innovation en bioprocédés industriels au Québec
- Novalait

Total budget: $282,866

Contact persons

Project supervisor:

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A targeted treatment for post-partum infertility in dairy cows

Duration: 2018-2021

Highlights

- One of the major problems in dairy production is related to the fact that cows have lower fertility in the two to three months following parturition, specifically high-yielding cows.
- New scientific data obtained in recent years suggest that the ovaries of dairy cows in the post-partum period are sensitive to the amount of available energy. An abnormal response has been observed at Day 60, particularly for genes that depend on Vitamin A.
- In a second study, 48 cows at Day 60 were categorized according to their metabolic deficit using their blood BHB levels to complete a comprehensive transcriptomic analysis of their dominant follicle. This analysis revealed a functional deficiency in Vitamin A as well as Vitamin D.
- As both vitamins are excreted into the milk and high producers show even greater losses, it is logical to assume that this deficiency is sub-clinical and that the cows' physiological needs may be greater during this period.
- Additionally, not every herd goes out to pasture (fresh grass), a situation that is known to affect fertility.
- The objective of this project is to test the addition of Vitamins A and D between Days 50 and 90 post-partum in conditions that are representative of Quebec dairy farms.
- The hypothesis is that the ovaries will sense a seasonal effect and that a targeted supplement of Vitamins A and D will reproduce the arrival of fresh pasture (Vitamin A) in spring (Vitamin D) and influence fertility.

Objectives

The objective of this project is to test the addition of Vitamins A and D between Days 50 and 90 post-partum in conditions that are representative of Quebec dairy farms.

More specifically, the objectives are to:

1) Determine whether the vitamin supplement corrects the ovarian response by analyzing the dominant follicle cells of the treated and untreated cows and according to BHB levels (high or low);
2) Determine whether the vitamin supplement improves the fertility levels of cows presenting high BHB levels at the start of the insemination period.

Results and potential benefits

This project proposes categorizing 48 cows on Day 50 based on their energy profile by measuring blood BHB levels at the farm and beginning supplementation for half of the animals (n=24), including 12 cows with a BHB level greater than 0.8. The second half of the animals (n=24) will not be treated in order to validate the therapeutic effect of the vitamins. To measure the response to the treatment, 24 cows (6 per group) will undergo aspiration of the dominant follicle to demonstrate the correction to the gene expression profile through supraphysiological vitamin supplementation. To better understand the therapeutic effect, a fatty acid profile will be completed on the follicular liquors, as well as a Vitamin A (retinol) analysis. Additionally, all animals will be monitored and inseminated to obtain preliminary results on the effectiveness of the treatment on fertility rates before attempting to reproduce the treatment on a commercial scale. The hypothesis is that ovaries sense a seasonal effect and that a targeted supplement of Vitamins A and D will emulate the arrival of fresh pasture (Vitamin A) in spring (Vitamin D) and thereby influence fertility.

Innovative aspects

- Use of genomic tools to understand the ovarian response to post-partum metabolic stress.
- Solution customized to each animal to maximize the effect of the vitamins by treating only those animals that need them at the time.
- Potentially more effective and acceptable than medical heat induction.

Professional trained

One Master's student was recruited in 2019, namely agronomy graduate **Martine Boulet**.

For further information

The research results will be promptly transferable to dairy farmers. An article will be written for the journal *Le producteur de lait québécois*. In addition, a presentation proposal will be submitted to CRAAQ for the Quebec Dairy Cattle Symposium (*Symposium des bovins laitiers*). Other communication activities (articles and presentations) are planned for collaborating users, including Novalait and Lactanet. A presentation will also be given at the Novalait Forum Techno.

Financial contributions

Special call for proposals in dairy production and processing (2016–2021):

- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Quebec consortium for industrial bioprocess research and innovation (CRIBIQ)
- Novalait

Total budget: $186,311

Contact persons

Project supervisor:

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Comparison of cows produced by *in vitro* fertilization and artificial insemination based on fertility, health, and productivity

Duration: 2020-2023

Highlights

- The use of in vitro fertilization (IVF) is steadily increasing in the dairy genetic material industry. A considerable proportion of bulls and cows of high genetic value today are the product of IVF.
- Currently, there is no phenotypical monitoring of these animals, although fertility data and some data on health and productivity are recorded by different stakeholders including Lactanet. As a result, there is no structured analysis for connecting the effects of IVF and the resulting phenotype despite the amount of data available on cattle and the data published on mice and humans.
- In those species, the phenotypic manifestations of IVF are strikingly similar to the manifestations observed and are in line with the mother's metabolic status and therefore suggest that cattle could respond to a metabolic program geared toward milk production.
- The purpose of the project is to match (based on genetics and environment) hundreds of animals produced by IVF and compare them to cows produced by artificial insemination based on productivity, fertility, and longevity (health) parameters.
- It will answer an extremely important question about the use of new reproductive technologies.

Objectives

The purpose of the project is to match (based on genetics and environment) hundreds of animals produced by IVF and compare them to cows produced by artificial insemination based on productivity, fertility, and longevity (health) parameters.

Results and potential benefits

In dairy cattle, the current goal of selection is to increase the average number of lactations per cow and therefore revenue by selecting cows with fewer health and fertility problems, such as infections and poor embryo quality. While we know that these traits have low heritability, recent Canadian estimates show variability between the extremes in the bull population, which offers a certain advantage for genetic improvement. In addition, this project aims to include epidemiological information in dairy cattle selection strategies to improve longevity and lifetime milk production. For genetics companies, knowledge of the presence or absence of a phenotypic effect arising from IVF is important for product development and improvement if there is a negative difference or for amplification if there is a positive difference. We must also consider that the phenotype that could arise from IVF is affected by environment and therefore could be adjusted by adapted conditions (e.g., limiting food a bit more) that mitigate the potential effects on production or reproduction.

With Boviteq, a phenotypic analysis of cows produced by IVF will enable the creation of subcategories according to the conditions present during IVF (e.g., age of the donor and bull, feed, culture medium, place of production, type of receiver) and the refining of production parameters that promote favourable phenotypes. Furthermore, producers could have the competitive advantage of having useful information on the phenotypic effects of IVF in order to make the best use of it in and outside Quebec.

Innovative aspects

- Use of a large-scale epidemiological approach
- Creation of epidemiological data on the phenotype of IVF dairy calves in terms of fertility, longevity and productivity

Professional trained

Doctoral student **Simon Lafontaine** was recruited for the project.

For further information

The results of the research will be quickly transferable to dairy producers, and an article will be written for the journal *Le Producteur de lait québécois*. In addition, a presentation may be given during the Quebec Reference Center for Agriculture and Agri-food's Quebec Dairy Cattle Symposium. Other outreach events (articles and talks) are planned for collaborators (e.g., Novalait, Lactanet and Boviteq), and a presentation will be given at the Novalait Forum Techno.

Financial contributions

- Natural Sciences and Engineering Research Council (NSERC)
- Quebec consortium for industrial bioprocess research and innovation (CRIBIQ)
- Boviteq
- Lactanet
- Novalait

Total budget: $154,210

Contact persons

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The benefits of using new, more digestible alfalfa for Quebec's dairy farms

Duration: 2018-2021

Highlights

- The general objective of the project is to evaluate the benefits of using more digestible alfalfa for Quebec dairy farms.
- The digestibility of the potentially more digestible alfalfa cultivars (conventionally selected) was similar to the controls. Compared to the controls, the cultivars that were genetically modified (GM) for increased digestibility did, in fact, had a higher in vivo digestibility of NDF fibre and a similar performance.
- The use of GM alfalfa cultivars could extend the harvest period and make it less subject to weather-related hazards while maintaining a comparable digestibility of NDF fibre. Compared to a control alfalfa harvested earlier, GM alfalfa harvested later had a higher yield and similar in vitro digestibility of NDF fibre, but a higher NDF fibre content and a lower crude protein content.
- The use of the potentially more digestible, conventionally selected alfalfa cultivar, (i.e., non-GM) had no effect on the in vivo digestibility of NDF fibre or on dairy cow performance when compared to that of a control cultivar.
- In addition, the animal test demonstrated that it is possible to reduce the supply of metabolizable proteins in the ration without negatively affecting dairy cow productivity at the beginning of lactation, provided the ration meets their needs for essential energy and amino acids. Such changes to the ration help lower the nitrogen emitted into the environment, thereby reducing the environmental impact of milk production.
- Based on our partial budget estimates, there is an economic interest in using GM alfalfa, especially if it is harvested to increase quality and the increase in cow production is associated with quota purchasing.
- Although the use of more digestible GM alfalfa may be cost-effective in certain scenarios, its use remains controversial in terms of social acceptability. The research team will produce an objective opinion on the performance of this alfalfa in the Quebec context.

Objectives

The general objective of the project is to evaluate the benefits of using more digestible alfalfa for Quebec dairy farms. The specific objectives included:

1) To evaluate a number of more digestible alfalfa cultivars and populations (CONV or GM) in Quebec's bioclimatic conditions for their yields, nutritional values and persistence (plant component);
2) To evaluate the impact of using a more digestible alfalfa cultivar as well as lower metabolizable protein content and variable energy intake on dairy cow performance (animal component);
3) To evaluate the technical and economic impacts of using this alfalfa cultivar on dairy farms in Quebec (technical/economic component).

Results and potential benefits

Plant component: All cultivars survived winter conditions well. Results from the first year of production show that all cultivars had comparable seasonal yields to the controls, except for the cultivar selected for improved enzyme degradability of the stem (-13%). The GM cultivars had an *in vitro* digestibility of NDF fibre (NDFd) of 4.7 percentage units higher and an NDF fibre content of 1.0 percentage units lower than the controls. The conventionally selected alfalfa cultivars had a similar NDFd to the controls. The GM cultivars also offered greater harvesting flexibility, making it possible to delay harvesting in order to increase yield while maintaining a level of digestibility similar to the controls. With one less cutting per year when harvested at the early flowering stage, the GM cultivars had a seasonal yield of 1.0 t DM/ha higher and a similar NDFd, but a lower crude protein content (-3.1 percentage units), higher NDF level (+5.6 percentage units) and a higher lignin content (+0.16 percentage units) than those of the control cultivars harvested at the early budding stage.

Results and potential benefits, suite

Animal component: The use of the potentially more digestible, conventionally selected alfalfa cultivar had no effect on the *in vivo* digestibility of NDF fibre or on dairy cow performance when compared to that of a control cultivar. It was also demonstrated that a reduced metabolizable protein supply is possible if the essential amino acid and energy needs are met in dairy cows fed alfalfa-rich diets. Compared to a control ration, decreasing the metabolizable protein supply and maintaining the same energy balance improved the efficiency of the animal's protein use by 21.5%, while maintaining energy-corrected milk production as well as the production of fat and true milk proteins. In addition, this ration resulted in a 24.0% decrease in nitrogen excretions. Such changes to the ration help lower the nitrogen emitted into the environment, thereby reducing the environmental impact of milk production.

Technical/economic component: When the GM alfalfa and the control alfalfa are harvested at the early flowering stage, the GM alfalfa is more digestible. In the economic analysis conducted in the partial budgets, two options were considered to manage the increased cow productivity generated by an increased ingestion of more digestible alfalfa, quota purchasing, or cow sale. In the quota purchasing scenario, the net profit of an average farm with corn silage would increase by $7,884/year ($80/cow/year) as a result of the use of GM alfalfa. At the time of the cow sale, net farm income would then increase by $3,769/year ($38/cow/year) as a result of the use of GM alfalfa. Harvesting of GM alfalfa may be delayed to allow for a better yield while maintaining a fibre digestibility similar to that of control alfalfa harvested earlier, but the crude protein content is then reduced and the NDF fibre content is increased. The annual net profit increase associated with the use of a GM alfalfa cultivar would be lower in this scenario ($731/year for the farm, $7/cow/year). GM alfalfa cultivars could be economically attractive when used to increase the digestibility of NDF fibre, and the additional net benefit is greater when increased cow productivity is associated with quota purchasing.

Innovative aspects

- The productivity and nutritional value of these potentially more digestible alfalfa cultivars was tested for the first time in the field in the Quebec climate.
- Only the GM cultivars were more digestible than the control cultivars.
- A cultivar that was conventionally selected and marketed as being more digestible was evaluated in cows but was not shown to be more digestible.
- In certain scenarios, the use of more digestible alfalfa would have economic benefits on Quebec dairy farms.
- It is possible to decrease the metabolizable protein supply while maintaining cow performance, thereby reducing nitrogen excretion and the environmental impact of dairy production.

Professionals trained

Master's students:

- **Marie-Soleil Boucher** (U. Laval, master's degree in plant biology with thesis, 2019-2021)
- **Jean-Philippe Laroche** (U. Laval, master's degree in animal science with thesis, 2018-2020)
- **Yatandi Djiguiba** (U. Laval, master's degree in plant biology, 2018-2020)

For further information

Lectures

- Boucher, M.-S.*, G.F. Tremblay, P. Seguin, É. Charbonneau, M. Thériault, J.-P. Laroche, A. Bertrand, A. Claessens, G. Bélanger, R. Berthiaume, and C. Halde. 2021. *Valeur nutritive, rendement et persistance de cultivars de luzerne (Medicago sativa L.) plus digestibles récoltés à différents intervalles de coupe sous les conditions bioclimatiques du Québec*. Master's seminar. April 13, 2021. Virtual format.
- Boucher, M.-S.*, and J.-P. Laroche*. 2021. *La luzerne, peut-elle être plus digestible et comment la valoriser ?* Seminar presented to the Quebec City Research and Development Centre, Agriculture and Agri-Food Canada. April 8, 2021. Virtual format.
- Boucher, M.-S.* 2021. *La luzerne peut-elle être plus digestible ?* Invited speaker at the Ordre des Agronomes du Québec–Québec City Section Déjeuner-Conférence. March 30, 2021 Virtual format. Yvon Lévesque research grant. Invited speaker.
- Boucher, M.-S.*, G.F. Tremblay, P. Seguin, É. Charbonneau, M. Thériault, J.-P. Laroche, A. Bertrand, A. Claessens, G. Bélanger, R. Berthiaume, and C. Halde. 2020. *Est-ce que les cultivars de luzerne potentiellement plus digestibles le sont vraiment et qu'en est-il de leur rendement sous nos conditions ?* Poster presented at the annual conference of the Centre SÈVE – Recherche en sciences du végétal. Nov. 25 and 16, 2020. Virtual format. 1st Prize at the 2020 Student Scientific Poster Competition.
- Boucher, M.-S.*, G.F. Tremblay, P. Seguin, É. Charbonneau, M. Thériault, J.-P. Laroche, A. Bertrand, A. Claessens, G. Bélanger, R. Berthiaume, and C. Halde. 2020. *Assessing performance of alfalfa cultivars with improved digestibility in Quebec, Canada*. Poster presented at the 11th Annual Canadian Forage and Grassland Association. Nov. 18 and 19, 2020. Virtual format.
- Boucher, M.-S.*, G.F. Tremblay, P. Seguin, É. Charbonneau, M. Thériault, J.-P. Laroche, A. Bertrand, A. Claessens, G. Bélanger, R. Berthiaume, and C. Halde. 2020. *Performance and digestibility of alfalfa cultivars developed for improved digestibility in Eastern Canada*. Poster presented at the American Society of Agronomy, the Crop Science Society of America, and the Soil Science Society of America International Annual Meeting. Nov. 9 and 13, 2020. Virtual format.
- Boucher, M.-S.*, G.F. Tremblay, P. Seguin, É. Charbonneau, M. Thériault, J.-P. Laroche, A. Bertrand, A. Claessens, G. Bélanger, R. Berthiaume, and C. Halde. 2020. *Les luzernes plus digestibles: le sont-elles vraiment et qu'en est-il de leur rendement sous nos conditions ?* Poster presented at the Symposium sur les bovins laitiers. Nov. 3 and 4, 2020. Virtual format.
- Laroche, J.-P.*, R. Gervais, H. Lapierre, D.R. Ouellet, G.F. Tremblay, C. Halde, M.-S. Boucher and É. Charbonneau. 2020. *Effet de la réduction des apports en protéines métabolisables dans des rations pour bovins laitiers équilibrées pour les acides aminés et ayant un niveau variable d'énergie*. Master's seminar. May 13, 2020 Virtual format.
- Laroche, J.-P.* 2020. *Nutrition protéique: Peut-on faire plus avec moins ?* Invited speaker, Déjeuner-Conférence de l'Ordre des Agronomes du Québec - Quebec City section. Quebec City, QC, Canada. Feb. 21, 2020. Yvon Lévesque research grant. Invited speaker.
- Boucher, M.-S.*, and J.-P. Laroche*. 2020. *La luzerne peut-elle être plus digestible et comment la valoriser ?* Oral presentation at the Journée laitière des partenaires organized by MAPAQ. Saint-Bruno (Lac-Saint-Jean), QC, Canada. Feb. 12, 2020. Invited speaker.
- Laroche, J.-P.*, R. Gervais, H. Lapierre, D.R. Ouellet, G.F. Tremblay, C. Halde, and E. Charbonneau. 2019. *Nutrition protéique: Peut-on faire plus avec moins?* Poster presented at CRAAQ's Dairy Cattle Symposium (Symposium sur les bovins laitiers), Drummondville, QC, Canada. Oct. 29. 2019. Alain-Fournier Award for the best student poster.
- Boucher, M.-S.*, and J.-P. Laroche*. 2019. *Essai de cultivars de luzerne plus digestibles et leur impact sur la production laitière*. Innovation day on forage plants and field crops at the Université Laval Station agronomique de recherche. Saint-Augustin-de-Desmaures, QC, Canada. July 3, 2019. 100 participants. Invited speaker.
- Halde, C.* 2018. *Quels gains pour les fermes laitières québécoises d'utiliser les nouvelles luzernes plus digestibles ?* General meeting of Novalait shareholders, Holiday Inn, Longueuil, QC, Canada. Nov. 1, 2018. Invited speaker.
- Halde, C.* 2018. *Visit of the more-digestible alfalfa project parcels*. Open house at the Station agronomique de recherche in Saint-Augustin-de-Desmaures, QC, Canada. July 25, 2018. 55 participants. Invited speaker.

Master's thesis

- Boucher, M.-S. (in progress). *Évaluation de la valeur nutritive, du rendement et de la persistance de cultivars de luzerne plus digestibles et témoins récoltés à différents intervalles de coupe*. Master's thesis. Université Laval, Quebec City (QC) Canada. Submission scheduled for 2021.
- Laroche, J.-P. 2020. *Effet des apports en protéines métabolisables et en énergie de rations à base de luzerne sur les performances de vaches en lactation*. Master's thesis. Université Laval, Quebec City (QC) Canada.

For further information, suite

Popular science articles

- Halde, C., G.F. Tremblay, P. Seguin, M.-S. Boucher, J.-P. Laroche, R. Gervais, A. Bertrand, A. Claessens, G. Bélanger, R. Berthiaume, H. Lapierre, D.R. Ouellet, M. Thériault, and É. Charbonneau. 2021. *The benefits of using new, more digestible alfalfa for Quebec's dairy farms* Factsheet distributed at the Novalait Forum Techno virtual conference. June 8–9, 2021. Forthcoming.
- Boucher, M.-S., G.F. Tremblay, P. Seguin, É. Charbonneau, M. Thériault, J.-P. Laroche, A. Bertrand, A. Claessens, G. Bélanger, R. Berthiaume, and C. Halde. 2021. *Les luzernes vendues comme étant plus digestibles ne le sont pas toutes !* Article in the Écho-Fourrager newsletter published by the Conseil québécois des plantes fourragères (CQPF). January 2021. Number 1. p. 7-8.
- Laroche, J.-P, R. Gervais, C. Halde, M.-S. Boucher, É. Charbonneau, H. Lapierre, D.R. Ouellet and G.F. Tremblay. 2020. *Des rations faibles en protéines pour améliorer l'efficacité des vaches laitières*. Article in the specialized journal *Le producteur de lait québécois*. Oct. 2020. p. 36-37.
- Laroche, J.-P. 2020. *Un apport en protéines diminué sans pénaliser la production laitière*. Article in the weekly publication La Terre de chez nous. May 20, 2020. P. A14.
- Bellavance, A.L., C. Halde, et G. Tremblay. 2017. *Vers une luzerne plus digestible*. Article in weekly La Terre de Chez Nous. Column published by the Conseil québécois des plantes fourragères (CQPF). La Terre de Chez Nous. July 12, 2017. p. A28.

Financial contributions

Special call for proposals in dairy production and processing (2016-2021):

- Quebec consortium for industrial bioprocess research and innovation (CRIBIQ)
- Novalait
- Natural Sciences and Engineering Research Council of Canada (NSERC)

Total budget: $177,996

Contact persons

Project supervisor:

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Hélène Lapierre
Agriculture and Agri-Food Canada

Daniel Ouellet
Agriculture and Agri-Food Canada

Philippe Séguin
McGill University

Gaëtan Tremblay
Agriculture and Agri-Food Canada

Could the fatty acid profile of milk help us detect and prevent ruminal acidosis in cows?

Duration: 2018-2021

Highlights

- Changes in milk production per cow over the last decades is partly due to an increase of feed concentrates in rations, but this increase could also be at the root of a metabolic disease found in dairy herds, namely sub-acute ruminal acidosis.
- This disease costs the North American dairy industry between US$500 million and US$1 billion per year, or US$1.12 (CA$1.44) per sick cow per day on average.
- The link between the fatty acids (FA) in milk and rumen health has been demonstrated, but the analyses used to establish this link cannot be used in fieldwork due to their prohibitive costs and the time required to perform them.
- Thanks to advances in infrared (IR) spectroscopy, we can now determine the FA profile of a milk sample in just a few seconds and at a fraction of the cost of analyses that use gas-phase chromatography (GC).
- If the results obtained are conclusive, the acidosis test could be part of Lactanet's offer and allow farmers to quickly and inexpensively obtain an overall picture of the risk of sub-acute ruminal acidosis in their herd.
- For Lactanet, this would be an effective way to valorize the analysis while making maximizing the information that can be derived from the milk samples during milk recordings.

Objectives

The main objective is to develop methods of detecting and preventing sub-acute ruminal acidosis in dairy herds. The project will respond to two specific objectives:

1) Develop a method of detecting sub-acute ruminal acidosis based on the FA profile of milk through IR spectroscopy using rumen pH readers. This will make it possible to establish links between the FA profiles of milk and rumen pH;
2) Determine the causes of sub-acute ruminal acidosis encountered throughout the project to better prevent the development of this metabolic disease in dairy herds.

Results and potential benefits

Currently, there is no simple and effective method of detecting acidosis in commercial dairy herds. The project took place on 12 commercial farms in the Lac St Jean region and allowed us to collect data to develop a method of detecting the disease. As part of the project, the FA profiles of milk samples from lactating cows over 1,000 days were analyzed, for a total of nearly 3,000 samples (two milkings per day and a composite sample of these two milkings). IR analyses were performed in Lactanet's laboratories, and the method is in the process of being validated through gas chromatography (GC) completed in Université Laval's laboratories.

The project will result in improved technical and economic performance on dairy farms as a decrease in the adverse effects of sub-acute ruminal acidosis combined with improved feed efficiency will serve to valorize feed. Consequently, improved feed efficiency would result in increased milk production and its components and therefore in increased revenues. Sub-acute ruminal acidosis can lead to other health problems that can require veterinary interventions and the use of medications, which then increases the cost of production and negatively affects the cows' well-being. Socially speaking, this project is perfectly aligned with the Dairy Farmers of Canada's "proAction" initiative that was launched in 2013, which focuses on cow comfort and well-being and highlights the importance of considering feed management to ensure their health and well-being. Environmentally speaking, reducing the incidence of sub-acute ruminal acidosis would hopefully improve cow longevity, which would reduce the number of replacement cows that must be bred and therefore reduce associated greenhouse gases (GHG) released in the form of enteric methane. Lactanet will make the expected results available as soon as possible at the end of the project to farmers, who will be able to quickly and inexpensively obtain an overall picture of the risk of sub-acute ruminal acidosis in their herd.

Innovative aspects

- Potential for faster detection of acidosis before damage is caused to animal.
- Potential for predicting ruminal pH based on fatty acid profiles determined using IR spectroscopy rather than chromatography, which can be carried out more quickly and at a fraction of the current cost.

Professional trained

One Master's student, **Félix Huot**, is in the process of receiving training at Université Laval's Department of Animal Science.

For further information

If the results obtained are conclusive, the acidosis test could be part of Lactanet's offer and become a new tool for monitoring individual cows. The research results will be transferable to dairy farmers in the very short term. Articles will be published in *Le producteur de lait québécois*, *Le Savoir laitier* and other popular science publications. Conferences for users will be held and posters will be created (Novalait Forum Techno and Symposium sur les bovins laitiers) in collaboration with our partners at Lactanet.

Financial contributions

Special call for projects in dairy production and dairy processing (2016-2021):

- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Consortium de recherche et d'innovation en bioprocédés industriels du Québec (CRIBIQ)
- Novalait
- 12 dairy farms from Saguenay-Lac-Saint-Jean Region

Total budget: $370,261

Contact persons

Project supervisor:

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Industrial research chair on sustainable life of dairy cattle: practices to improve cow comfort and welfare

Duration: 2016-2021

Highlights

- Dairy farmers face the challenge of providing consumers with high-quality products that meet societal expectations for environmental sustainability, animal welfare, and economic accessibility, while ensuring increased productivity based on an understanding of the nutritional and physiological needs of dairy cows.
- Increasing the lifespan of dairy cattle raised in housing in an environment that promotes their comfort and health is a sustainable solution that increases the profits generated by companies, limits their environmental footprint, and addresses concerns about animal welfare by reducing involuntary culling.
- For these reasons, and to develop expertise in this field, the Industrial Research Chair on Sustainable Life of Dairy Cattle was created at McGill University on January 1, 2016. This major research initiative aims to provide dairy farmers with concrete data and tools to optimize comfort and increase the life span of dairy cows, with a particular focus on tie-stalls.
- The Chair also aims to improve recommendations for animal welfare to help farmers prepare for the implementation of the animal welfare component of the ProAction® national program.
- The Chair's work has produced results on changes in stall configuration to increase comfort and opportunities for movement for cows in tie-stalls. The following studies address the impact of access to exercise on cow behaviour, welfare, and health.
- The second research theme will examine long-term profitability measures and herd longevity. The projects are conducted through a database analysis of Quebec dairy herds.

Objectives

- Optimize cow comfort and increase opportunities for movement, specifically cows in tie-stalls, by modifying housing and access to exercise.
- Increase cow longevity while taking dairy farm profitability into account.

Results and potential benefits

The Chair's research activities focused on two main themes:

Cow comfort and herd management

- New knowledge has been developed on behavioural measures, which can be used to automate the monitoring and detection of problems related to the welfare of cows in tie-stalls. For example:
  - A 3-D pedometer (IceTag) accurately measures the number of steps taken by the cow in its stall (Shepley et al. 2017 Agriculture 7:53)
- A number of studies have been conducted to assess the impact of housing on movement opportunities and cow comfort.
  - Cows use the extra space provided to them: all the characteristics of the stall have the potential to provide additional space, whether by increasing the length of the chain, or the width or the length of the stall. We confirmed that bedding is an essential element of comfort (the more, the better) and that hock injuries heal in 6 weeks when cows are housed in stalls with deep bedding (St-John et al., 2021 JDS 104): 3304; Boyer et al., 2021a JDS 104:3316; Boyer et al., 2021b JDS 104:3327; McPherson and Vasseur, 2021 JDS 104:3339).
  - What movement opportunities can be offered to cows outside the stall? We found that housing dairy cows in enclosures during the dry-off period was beneficial for rest and recovery with respect to locomotor abilities (Shepley et al., JDS 102): 6508; Shepley et al., in revision). The next studies focus on access to exercise for dairy cows in tie-stalls and its impact on the cow behaviour, welfare, and health (specifically locomotor). Analysis of the results is in progress.

Results and potential benefits, suite

Dairy cattle longevity

- We analyzed the impact of the first incidence of mastitis or lameness on performance, profitability, and longevity at different stages of lactation on 20,000 cows in 120 Quebec herds:
  - We found that, during the first lactation, a first case of mastitis or lameness reduces production and profit by up to 1,200 kg and $1,000 CAD per sick cow while increasing the risk of culling up to 2x (Puerto et al., 2021a. JDS.2020-19584; Puerto et al., 2021b. JDS.2020-19585);
  - Lactation results suggest that the revenues generated in the current lactation have largely influenced the retention capacity of cows in the herd for another lactation. Profitable cows appeared to be more efficient with respect to feeding until lactation 2 and less likely to contract diseases during subsequent lactations. These findings have led to the development of an interactive decision-making support tool that leverages information on costs and cumulative lifetime revenues to assess herd profitability (Warner et al. under review);
- In collaboration with the Lactanet R&D team, we used Valacta databases to remotely detect low-welfare herds:
  - The model worked well for lameness but less well for hock injuries (Warner et al., 2020. COMPAG 169: 105193);
  - We developed a tool for comparative welfare analysis (Herd Status Index HSI) based on 14 indicators derived from routine dairy control data (Warner et al., 2020 Animals 10: 1689).

Professional trained

The Research Chair participates in the training of highly qualified personnel. Since its inception, 48 people have been trained and actively involved in projects.

PhD: **Gabriel Machado Dallago** (in progress) and **Elise Shepley**

Master's degree with thesis: *in progress*
Anna Bradtmueller
Amir Nejati
Rachel Chiasson
Catherine Lussier
Naziya Mauyenova

Jordan Tonooka
Maria A. Puerto Rodriguez
Sarah McPherson
Véronique Boyer
Jessica St John

Applied master's degree:
McGill
Oluwaseyi Adeboye
Sirine El Hamdaoui

VetAgroSup Clermont, France
Nicolas Gafsi
Anthony Pic

Université de Sassari, Italie
Maria Francesca Guiso
Giovanni Obinu

ISA Lille, France
Manon Demaret
Sirine El Hamdaoui

AgroCampus Ouest, France
Géraud Plas-Debecker
Marianne Berthelot

Postdoctoral fellows:
in collaboration with Lactanet
Maxime Leduc
Daniel Warner
Liliana Fadul Pacheco

Hector Delgado

For further information

- Several articles reporting the progress of the Chair's work were published in the journal *Le producteur de lait Québécois*.
- Poster presentations at the 2018 and 2021 and Novalait Forum Techno
- Chair website: mcgill.ca/animal
- Chair blog: cowlifemcgill.com
- Chair Twitter account: @CowLifeMcGill
- 3 short videos were produced: video 1 video 2 video 3

Financial contributions

The Research Chair is funded through the Natural Sciences and Engineering Research Council of Canada (NSERC) Industrial Research Chairs program. Industrial partners include:

- Novalait
- Dairy Farmers of Canada
- Lactanet

McGill University has also provided financial contributions.

Total budget: $1,720,000

Contact persons

Project supervisor:

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René Lacroix
Débora Santschi
Daniel Lefebvre
Lactanet team

Educational leadership chair in sustainable agricultural building design

Duration: 2018-2023

Highlights

- Between 2008 and 2015, capital spending in Quebec's livestock production assets fell from $475M to $300M.
- Major reinvestments were made beginning in 2017 ($500M/year) to modernize livestock production facilities and boost their competitiveness.
- In order to support these investments, Quebec needs skilled labour capable of providing sound advice to the province's roughly 10,500 farmers.
- The Chair will train engineers and agronomists specializing in quality and competitive infrastructure, integrating the latest standards in animal welfare and environmental protection.
- Research projects will focus on new sustainable concepts for animal production such as sustainable grazing methods for dairy cattle, low-emission aviary systems for laying hens and advanced ventilation systems for broiler chickens and hogs.
- The Chair's activities will provide up-to-date technical documentation to help farmers make informed investment decisions.
- Optimizing farm building design and the efficient use of agricultural equipment could help cut annual infrastructure costs by 1%.

Objectives

The Chair aims to support training, research and knowledge transfer in animal production engineering. With a view to ensuring the long-term sustainability of Quebec's livestock facilities, research will focus on improving animal welfare, reducing farms' environmental impact and improving farm competitiveness. Knowledge and skill development for future university graduates as well as concerned stakeholders revolves around the following four pillars:

- Advanced production systems;
- Advanced environmental control;
- Optimal management of resources and waste;
- Energy efficiency and alternative energy sources.

Results and potential benefits

The Chair will provide training to engineers and agronomists specializing in quality and competitive infrastructure, integrating the latest standards in animal welfare and environmental protection.

The Chair will also be an opportunity to experiment, with support from graduate students, with new concepts in sustainable dairy production such as sustainable grazing areas, solar roofs on stables and recycled manure bedding (RMB). The objective of the first project on raised terraces is to provide an opportunity for movement at any time of the year that is more cost-effective than tie-stalls. This is also a more acceptable option than wintering areas from an environmental standpoint. The goal of the second project on greenhouse-stables is to verify if the additional costs of this type of building are offset by the benefits that more natural, ambient conditions provide. The third project on RMB aims to demonstrate that biomethanization can generate a hygienized, pathogen-free digestate with the right physical characteristics to ensure the health and well-being of cows.

The Chair also intends to produce new tools that will be available on a web-based platform for all farmers and stakeholders in the sector.

In this regard, all activities aim to provide up-to-date information to help the province's roughly 10,500 livestock producers (dairy cattle, hogs, poultry, sheep and goats) make informed investment decisions when upgrading their facilities. Optimizing building design and the efficient use of animal production equipment could help cut annual infrastructure costs by 1%. Considering annual capital expenditures in the order of $500M, this would represent savings of $25M over five years.

Innovative aspects

- The Chair is the only academic institution in Quebec and Canada dedicated to training and knowledge transfer as related to agricultural buildings.
- The Chair is a catalyst of research projects focusing on livestock facilities.

Professionals trained

- **Alexandre Blouin**, **Ève-Marie Houde** and **François Savard** (B. Eng. – Agro-environmental engineering): Comparison of the economic and environmental performance of conventional stables and greenhouse-stables.
- **Zakary Picard** (B. Eng. – Agro-environmental engineering): Assessment of the environmental and energy performance of ventilation systems in livestock buildings.
- **Béatrice Dupont-Fortin** (B. Eng. – Agro-environmental engineering): Assessment of animal behaviour and well-being in outdoor exercise areas.
- **Andrea Katherin Carranza Diaz** (PhD – Soils and Environment): Development of simplified methods for measuring air-borne contaminants in livestock buildings.
- **Paz Elizabeth Álvarez-Chávez** (PhD – Soils and Environment): Demonstration of environmental and economic efficiency of the raised terrace concept as a sustainable grazing method for tie-stalled dairy cattle.
- **Sebastian Gutierrez Pacheco** (PhD – Ecological engineering): Instrumentation and controls in agriculture and precision breeding.

For further information

- Presentation of students' work at the Chair's annual meetings.
- Creation of a website and a Facebook page dedicated to the Chair's work.
- Presentations at local (e.g. Forum Novalait and the Symposium sur les bovins laitiers) and international (e.g. International Commission of Agricultural and Biosystems Engineering) events.
- Publication of general science articles in agricultural journals (e.g., *Le Bulletin des agriculteurs*).
- Participation in the organization and content development of online continuing education courses and Open House events organized by partners.

Financial contributions

The Chair is funded by a number of organizations and engineering/equipment consultants.

- Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ)
- Novalait
- Union des producteurs agricoles du Québec (UPA)
- Institut de recherche et de développement en agroenvironnement (IRDA)
- Université Laval, Department of Agriculture and Food Sciences
- Industries Harnois
- Lactanet
- Équipements Jolco
- Consultants Lemay & Choinière
- Consumaj
- Les Consultants Mario Cossette
- Groupe Alco
- Intelia
- Maximus
- Zaxe Technologies
- Association des ingénieurs en agroalimentaire du Québec (AIAQ)

Total budget: $923,000

Contact persons

Project supervisor:

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Recycled manure bedding: recommendations for safe use to ensure milk quality

Duration: 2017-2020

Highlights

- There is growing interest in using recycled manure as bedding (RMB) for dairy cattle.
- It is therefore imperative to assess any disadvantages that this practice might cause on animal health, human health and milk quality.
- The objective of this research project was to establish recommendations for the safe use of RMB.
- The material and data required to complete the project were obtained from a study conducted on 27 dairy herds using RMB and 61 herds using straw bedding for comparison.
- The bacteriological quality of RMB before it is used varied from one production method to another and was generally lower than that of straw. Different microbial pathogens for cows and humans (*Cryptosporidium* spp., *Salmonella* spp.) were found more frequently in RMB than in straw. These microorganisms were never found in bulk tank milk, however.
- The number of cases of clinical mastitis was not any higher on farms using RMB; however, such cases were more often caused by *Klebsiella* spp., an agent that triggers very acute mastitis often resulting in the death or culling of the animal.
- Use of RMB did not affect pasteurization-resistant bacteria or spore counts in the milk, though the populations of resistant bacteria were different in milk from farms using RMB compared to those using straw.

Objectives

The project's specific objectives are:

- To describe the various bedding production processes currently used on farms, as well the bedding's bacteriological and physicochemical characteristics;
- To assess the impact of the RMB on cow hygiene and comfort and on mammary gland health;
- To verify the influence of the production and management of RMB on microbial populations present in the mammary gland;
- To analyze the impact of using RMB on the microbiota of bulk tank milk;
- To determine the survival rates of cryptosporidiosis and coccidiosis and the risk of transmitting the diseases through recycled bedding.

Results and potential benefits

Although commendable from a sustainable development perspective, the use of RMB in dairy farming presents a number of drawbacks in terms of animal and human health.

Indeed, our results demonstrated that several pathogens are capable of surviving current farm production methods. In certain cases (e.g. *Cryptosporidium* spp.), using RMB could potentially even magnify the transmission cycle within a herd. However, these pathogens were never found in bulk tank milk, which indicates that the measures in place at the farm's critical control points during milk collection are sufficient to control most of the risk for consumers. On the other hand, farm workers could be at greater risk and should therefore adopt strict hygiene measures (gloves, hand washing) when handling RMB.

Our results also suggest that this type of bedding should not be used for animals under the age of six months, which are particularly sensitive to these pathogens.

The number of cases of clinical mastitis was not higher on farms using RMB; however, such cases were more often caused by *Klebsiella* spp., an agent that triggers very acute mastitis often resulting in the death or culling of the animal.

Furthermore, in light of the higher severity of mastitis cases observed, the use of RMB could potentially compromise the well-being of the animals if other mitigation measures are not implemented.

Lastly, although bacteria populations found in milk from farms using RMB vary, the expected impacts on dairy processing appear to be minor at the present time, especially in the case of unripened cheeses.

Innovative aspects

- Completion of project on commercial farms using recycled manure for bedding.
- Analysis of the microbiological aspect of using recycled manure bedding on milk quality.
- Identification of bacterial profiles responsible for the development of clinical mastistis on farms using recycled manure.

Professionals trained

- **Annie Fréchette** (PhD), expertise in veterinary epidemiology at Université de Montréal.
- **Alexandre Jules Kennang Ouamba** (PhD), expertise in food science at Université Laval.
- **Mérilie Gagnon** (PhD), expertise in food science at Université Laval.
- **Marlén Irlena Lasprilla Mantilla** (Master's), expertise in parasitology at Université de Montréal.
- **Jessika Beauchemin** (Master's), expertise in veterinary microbiology at Université de Montréal.

For further information

The research results will be transferable to dairy farmers in the very short term (i.e., once the research project is completed). Articles will be published in *Le producteur de lait québécois*, *Le Savoir laitier* and The *Milk Producer*, and training sessions and conferences for users will be held (Novalait Forum Techno and Symposium sur les bovins laitiers) in collaboration with our partners at Lactanet and as part of the transfer activities of the FRQ-NT Op+Lait strategic cluster.

Financial contributions

Partnership for innovation un dairy production and dairy processing (EPI2015-2020):

- Consortium de recherche et d'innovation en bioprocédés industriels du Québec (CRIBIQ)
- Fonds de recherche Québec – Nature et technologies (FRQNT)
- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Novalait
- 92 dairy farms

Total budget: $318,839

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How do milk's natural microbiota and composition contribute to cheese quality?

Duration: 2018-2023

Highlights

- Cheese quality depends on multiple factors, including herd management on the farm, control of the cheese-making technology used and the microbiota present in the cheesemaking environment.
- The natural microbiota, also called microflora, of cheeses can come from many sources, but the most influential sources have not been identified to date.
- The effect of the choice of sanitizers on the composition of milk's natural microbiota and its persistence in the dairy industry is unknown.
- The protein and mineral composition of milk varies according to the season and influences its cheesemaking performance.
- Dosing techniques for two natural antimicrobial systems (lysozyme and lactoperoxidase) involved in dairy production have been developed and will be applied to cheese analyses.

Objectives

The primary objective is to gain a deeper understanding of milk's microbiological and chemical elements that influence cheese quality. More specifically:

- To monitor and characterize the natural microbiota of dairy environments, from the farm to cheese;
- To determine the effect of the natural microbiota on cheese texture;
- To measure the impact of concentrated systems, mineralization and natural antimicrobial systems on proteolysis and evolution of the microbiota during cheese ripening: relationship with rheology.

Results and potential benefits

At the end of this project, we aim to provide recommendations to dairy farmers and processors on the influence of milk's natural microbiota on the production of aromatic compounds. We also hope to provide information on desirable and undesirable secondary species in milk affecting the organoleptic qualities of cheeses. More specifically, a number of cheesemakers are currently in the process of collecting data that will support the addition of value-enhancing terms to their product labels (*Appellation d'origine*, *Appellation de spécificité*...) as the demonstration of uniqueness must be based on sound scientific evidence. Ultimately, we hope to identify microbial signatures related to different cheesemaking regions.

The results of this research project will also provide a better understanding of the impact of demineralization on cheese texture during ripening. Texture changes are generally attributed to proteolysis, but this effect is confused with demineralization. The proposed experimental approach will make it possible to establish the respective contributions of the two phenomena.

A comparative study of the physicochemical properties and cheese performance of summer and winter milks will also be conducted. This work aims to identify the causes of seasonal variations and propose appropriate technological solutions.

Lactoperoxidase and lysozyme remain active, even after pasteurization, and their content in cheese is proportional to the amount of serum that they contain. Lactoferrin seems to be slightly concentrated in cheese which suggests that antimicrobial systems may affect the microbiota during ripening. Milk standardization could then help to modulate the ripening microbiota.

Two types of cheese will be studied: Cheddar and washed rind cheeses. A total of eight cheesemakers will be participating in this research project, four of which process more than one million litres of milk per year and four of which process less than one million litres per year.

Innovative aspects

- Better understanding of concentrated dairy systems involved in cheese production.
- Better understanding of seasonal effects on milk's cheesemaking performance.
- Determination of the impact of sanitizing agents on cheeses' natural microbiota.
- Determination of the effect of natural antimicrobial systems on the evolution of certain bacteria that could influence texture during maturation.

Professionals trained

2 master's students:

- **Karl Coulombe**: Influence of the natural microbiota on cheese texture
- **Rachel Langlois-Deshaies**: Effect of seasons and milk ultrafiltration on the content of natural antimicrobial systems in cheese

1 PhD student:

- **Typhaine Morvant**: Detecting indigenous microorganisms and sensitivity to sanitizers

For further information

Results will be communicated at local scientific conferences and symposiums, including the STELA Colloquium, as well as national (Forum Novalait, Journées fromagères CEFQ) and international (World Dairy Summit FIL-IDF, Cheese symposium) conferences. We have already been invited and successfully presented the project to staff at the cheese factories and we plan to continue to do so.

Financial contributions

This project is funded as part of the Dairy Research Cluster 3 through Agriculture and Agri-Food Canada's Canadian Agricultural Partnership, Novalait and is administered by Dairy Farmers of Canada.

Total budget: $682,570

Contact persons

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Occurrence and impact of microbial biofilms on milk quality from farm to cheese vats

Duration: 2018-2023

Highlights

- Bacteria and biofilms are an inherent risk in cheesemaking that must be managed on a continuous basis. Biofilms, protective cultures that form on cells, can either be beneficial or harmful and can contain infectious bacteria. As a result, identifying and controlling the formation of biofilms is critical to ensuring and maintaining milk quality.
- The project's objective is to study milk biofilms, from the farm to dairy plants.
- The parameters to be studied include the origin, structure and composition of biofilms, and whether they are harmful or beneficial.
- Sixty dairy farms located in Quebec and Ontario, two industrial cheesemakers and two artisanal cheesemakers will be sampled to characterize the biofilms on a farm-to-plant continuum.
- The research team will use bioreactors to study the formation of milk biofilms.
- Microorganisms from the biofilms that form will be isolated and identified, and the microbial communities found in the farms and dairy plants will be compared.
- Bioprotective cultures will be characterized with the aim of preventing and eliminating the biofilms.
- The project will result in recommendations on managing milk quality and sanitizing equipment, in addition to increasing our understanding of the beneficial biofilms associated with quality dairy products.

Objectives

The project's main objective is to gain a deeper understanding of the milk biofilms present from the farm to dairy plants.

Specifically, the research hypotheses are as follows:

1) Studying the ecology of biofilms from the farm to dairy plants will help determine the origin of the microorganisms that they contain and assess their impact on milk contamination;
2) A more complete characterization of dairy biofilms will allow for the development of quality control strategies;
3) Lactic acid bacteria can inhibit or promote the production of dairy biofilms.

Results and potential benefits

The initial findings will make it possible to compare the microbial communities involved in the formation of biofilms on farms and in dairy plants and generate knowledge of the impact of management practices on the appearance of biofilms. To date, it has been observed that there is a microbial biomass that adheres to farming equipment stronger than industrial cheesemaking equipment. This biomass is a complex ecosystem made up not just of bacteria, but also yeasts and moulds. Recommendations on milk quality management and equipment sanitation practices will also be issued to farmers and cheesemakers. In addition, a study on beneficial biofilms, both at the artisanal and industrial scales, will allow the sector to better characterize the microbial communities associated with product quality, including cheeses. Potential benefits:

- **Economic**
  - Reduced losses of milk and cheese related to biofilm contamination.
  - Production of higher quality dairy products.
  - Increased productivity due to targeted cleaning strategies.
- **Environmental**
  - Reduced losses of milk and cheese related to biofilm contamination.
  - Reduced use of chemical disinfectants and increased use of natural antimicrobials.
- **Social**
  - Consistent, high-quality dairy products.
  - Reduced presence of pathogens in dairy products.

Innovative aspects

- Study of the farm-to-plant continuum.
- More complete characterization of dairy biofilms due to advanced culturomics and high-throughput sequencing techniques.
- Use of natural antimicrobials to prevent or eliminate dairy biofilms.

Professionals trained

- **Samuel Jean**, MSc in Food Science, Université Laval and Agriculture and Agri-Food Canada; Farm management practices and agri-food microbiology
- **Carine Diarra**, MSc in Food Science, Université Laval; Parameters dictating biofilm formation
- **Jacob Vanderkoov**, MSC in Food Science, University of Guelph; Biofilm microbiology
- **Nissa Niboucha**, PhD in Food Science, Université Laval; Microbiology of biofilms in dairy processing
- **Coralie Goetz**, Postdoctoral fellow, Université Laval
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- **Caroline Chénard**, Research professional, Université de Montréal

For further information

A technological workshop on the study of biofilms was organized for members of the Op+lait research group on October 28, 2020. When the initial results are available for transfer to dairy farmers, a popular science article will be published in the journals *Le producteur de lait québécois* and *Milk Producer*. Scientific articles will also be published in peer-reviewed journals. Other communications activities (articles, presentations and workshop) have been completed and are planned for collaborating users, including Novalait, Lactanet, Dairy Farmers of Ontario, and more. A presentation will also be given at Novalait's Forum Techno.

Financial contributions

This project is funded as part of the Dairy Research Cluster 3 through Agriculture and Agri-Food Canada's Canadian Agricultural Partnership, Novalait and Dairy Farmers of Ontario and is administered by Dairy Farmers of Canada.

Total budget: $1,514,310

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The naturalness of food products – a multimedia approach for better understanding consumers' perceptions and behaviours

Duration: 2019–2022

Highlights

- In recent years, consumers have shown a growing interest in so-called natural products (Amos et al., 2014; Rozin, 2005; Siipi, 2013), which are associated with health and healthy food.
- Since the concept of naturalness is not clearly defined, it is important to understand how businesses operating in the food industry convey information on naturalness to consumers, how consumers perceive it, what factors influence those perceptions, and what effects these processes have on their behaviour.
- To answer these questions, a scale for measuring naturalness was created. An experimental study of 120 participants was then conducted to identify the primary determiners of the perceived naturalness of 20 products from 4 different categories: milk products and alternatives, meat, grain products, and fruits and vegetables.
- To measure the effect of various information on perceived naturalness that is placed on packaging, the number of visual or written references to "natural, organic, authentic, fresh, local, quality, (organic, non-GMO, local) certifications, and claims," as well as to the number of ingredients resonating as chemical or non-chemical were noted for each instance of packaging in the study.
- The regression analysis indicates that the number of references to organic nature and certifications has a positive effect on perceived naturalness, whereas the number of ingredients with chemical resonance has a negative effect, regardless of the product category.
- The results also show that for a positive effect on perceptions of a product's naturalness, consumers must find the information credible and logical.

Objectives

The study's three major objectives are as follows:

1) Study the psychological processes that occur when consumers are exposed, through the information on packaging, to food products associated with naturalness
2) Understand the relationships between strategies for communicating the naturalness of food products through packaging, the psychological processes involved when consumers are exposed to such information, and the behaviours that result
3) Assess the effect of Health Canada's new labelling regulation on perceptions related to the naturalness and health value of products

Results and potential benefits

The study results have led to a number of benefits:

1) Creation of a scale for measuring naturalness that is easy for suppliers to use to assess the perceived naturalness of a product prototype or to compare the perceived naturalness of various products from competitors
2) Identification of informational attributes that matter most for perceived naturalness—organic nature, certifications, and ingredients that resonate as chemicals—can guide suppliers when creating their products, but above all, it can inform how that information is conveyed on packaging. The communication of information on packaging is increasingly important in a pandemic when more consumers are shopping online
3) Regression analyses show that responsible packaging—that is recyclable, reusable, or compostable—positively influences the perceived naturalness of a product, which in turn influences the perceived healthiness of the product and ultimately consumers' intent to buy the product

As a result, better alignment between the information about naturalness that is conveyed by packaging and products' ingredients may have a positive effect on sales and ultimately encourage consumers to adopt behaviours that will improve their health.

Innovative aspects

- Creation of a scale for measuring the perceived naturalness of products
- Experimental study in a store where participants can handle products to assess them based on various aspects (e.g., quality, naturalness and healthiness). A comparison was then made with same participants' assessments based on photos of the same products' packaging
- Identification of key information on packaging that relates to the perceived naturalness of products

Professionals trained

- **Clara Dutrevis**, M.Sc., ESSEC Business School (ESSEC): Master's thesis on the development of a scale measuring the naturalness of food products
- **Stéphanie Lessard**, Ph.D. student, Université Laval: Supervised the collection of data from the experimental part of the study
- **Geneviève Groleau**, M.Sc., HEC: Literature review on naturalness, codification of information related to naturalness on product packaging, and statistical analyses
- **Helena Armengau-Ribeiro**, M.Sc., HEC L: Supervised M.Sc. project on the evolution of perceptions of different categories of products (dairy, meat, breads and cereals, fruits and vegetables) and the evolution of attitudes (e.g., health and the environment) (new data collected last December) and will conduct comparative analyses using the data from the first collection in November-December 2019. This will show how perceptions of product categories have evolved since the beginning of the pandemic.

For further information

Transfer to industry partners: The results were presented to corporate partners of the research project on December 9 and 10, 2020. Some businesses asked for personalized presentations. In addition, a talk will be given at Novolait's Forum Techno. The students involved in the project will write articles.

Financial contributions

RITA Recherche Innovation Transformation Alimentaire: a consortium mandated by MAPAQ at McGill University in collaboration with CTAQ and MEI. Below is the total budget for the 13 activities of the RITA network.

Budget total : $2,765,828

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METABIOLAC industrial research chair in metabolic activity and the functionality of bioprotective lactic cultures

Duration: 2016-2021

Highlights

- Market trends limit resorting to traditional microbiological barriers (e.g., chemical additives) to ensure food quality and safety.
- The discovery of new generations of natural and safer antimicrobials represent one of the most promising approaches.
- As part of this project, unique lactic acid bacterial strains with antibacterial and antifungal properties against pathogenic strains or spoilage strains in fermented dairy products were isolated and characterized.
- Technological processes were developed for the production and long-term stabilization of bioprotective cultures and functional ingredients.
- The effectiveness of the various ingredients developed was validated in different dairy matrices, notably cheddar (activity against *Clostridium tyrobutyricum*) and yogurt (antifungal activity).
- The effectiveness of bactofencin, nisin and reuterin against the clinical isolates responsible for bovine mastitis was demonstrated *in vitro*. In addition, the bacterial load of dairy cows' teat skin was reduced following the implementation of teat baths based on a combination of these three antimicrobial compounds.

Objectives

Our specific objectives in dairy sector are:

- To conduct *in vitro* and *in vivo* evaluations of the potential of new, natural antimicrobial-based products produced by lactic acid bacteria as an alternative to antibiotics in the prevention and treatment of sub-clinical and clinical mastitis in dairy cows;
- To develop an environmentally friendly approach based on the use of bioprotective lactic acid bacterial cultures or their metabolites for the positive modulation of microbial ecosystems in milk and dairy products and for controlling undesirable flora in these products. The impact of such an approach on the quality and safety of these products will be evaluated.

Results and potential benefits

Protective cultures and metabolites demonstrating unique antibacterial and antifungal activity: Several bioprotective lactic acid bacterial strains were identified and selected for their antibacterial or antifungal activity against pathogenic flora and spoilage flora in fermented dairy products. Some of these strains demonstrated an ability to inhibit the growth of moulds frequently isolated in cheese (*Penicillium chrysogenuym*) or a spoilage strain in dairy products such as *Clostridium tyrobutyricom*, which is responsible for the butyric swelling of cheese. Six strains of *Lactobacillus reuteri* that produce reuterin, a broad-spectrum aldehyde that can inhibit the growth of moulds in yogourt, were isolated. The purified reuterin obtained demonstrated strong fungicidal activity as well as antibacterial activity against pathogenic strains resistant to some antibiotics. This antimicrobial, as well as bactofencin and nisin, prevented the growth of bacteria isolated from bovine mastitis cases. The use of teat baths based on a combination of the three antimicrobial compounds reduced the bacterial load of dairy cows' teat skin, suggesting their potential use for the prevention of mammary infections in dairy cattle. In parallel, a fermentation and spray-drying process for the production of natural bioingredients, concentration and long-term stabilization of culture environments containing selected protective strains and their metabolites was developed.

Galenic food-grade forms for the protection and controlled release of bacterial cultures or their metabolites: Capsules for protecting protective cultures and metabolites with antibacterial and antifungal activity and gradually releasing them in food were developed and characterized. Their effectiveness as bioconserving agents was demonstrated in Cheddar cheese, grated Mozzarella cheese and stirred yogurt type models.

In the mid-term, this work will help improve the safety of dairy products through the use of natural products and will help reduce losses related with their downgrading. This new generation of safe, natural products might also be useful for stemming the apparition of mastitis in cows.

Innovative aspects

- Approach focusing on the use of new synergic consortiums based on bioprotective lactic acid bacteria and/or natural antimicrobial compounds in order to reduce the use of chemical additives and salt for food preservation (biofood sector) and antibiotics for the prevention of bovine mastitis (veterinary sector).
- Updating of innovative, industry-transferable processes for the small- and large-scale production, stabilization and protection of the various consortiums of antimicrobial compounds developed.

Professionals trained

Training of skilled workers in the field of milk and dairy product quality and safety.

Laurent Dallaire (entrepreneur, Innodal), **Hélène Pilote Fortin** (food specialist) and **Samantha Bennet** (research assistant) completed their Master's project in the context of the Chair, while **Sabrine Naimi** (postdoctoral fellow, Institut Cochin) and **Hebatoallah Hassan** (assistant instructor) completed their PhD studies. Additionally, three PhD students (**Liya Zhang**, **Samira Soltani** and **Isabelle Iachella**) are currently pursuing their research projects.

For further information

- Presentation of the results at Novalait's Forum Techno and annual meeting, as well as Agropur's annual scientific meeting and the Acfas's 88th annual conference
- Presentation of posters at national and international conferences
- Publishing of scientific articles in *Food Control*, *Frontiers in Chemistry*, *Frontiers in Microbiology and Probiotics and Antimicrobial Proteins*, *Industrial Biotechnology*, *ACS Infectious Disease*, *International Journal of Food Microbiology*, *Scientific Reports*, *Archives of Microbiology*, *FEMS Microbiology Reviews*, and *Environmental Microbiology*.
- Patenting
- Development of a spin-off (Innodal) by a chair student for the large-scale production of bioprotective cultures
- Signing of an agreement with a company to conduct research on the bioprotective activities of the *Carnobacterium divergens* M35 strain (approved by Health Canada as a new food additive) in fermented dairy products
- Tests for scaling up four bioprotective cultures were completed by a ferment company

Financial contributions

The research chair is funded under the program of the National Sciences and Engineering Research Council of Canada (NSERC) Industrial Research Chair in collaboration with the Quebec consortium for industrial bioprocess research and innovation (CRIBIQ).

Industrial partners are:

- Biena
- Cascades
- La Coop Fédérée
- Fumoir Grizzly
- Novalait
- Olymel
- Sani-Marc Group

Total budget: $1,916,685

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New approaches to convert carbohydrate-rich by-products (whey and milk permeate) into high added value products and contribute to the sustainability of the dairy industry

Duration: 2019-2022

Highlights

- World production of whey is estimated around 200 million tons per year with an increase rate of about 2%/per year. Implementing chemical and enzymatic transformations as a platform of whey conversion into added value by-products is expected to conciliate the needs of product diversification with the sustainable development.
- This research activity focuses on the development of the scientific and technological bases of the new approaches to value the lactose rich by-products (whey permeate and milk permeate) in products with high value, including the hydroxymethylfurfural (HMF), 2,5-furandicarboxylic acid (FDCA) lactosucrose and lactobionic acid.
- The US Department of Energy has listed 2,5-furandicarboxylic acid (FDCA) amongst the top-12 molecules with the potential to replace petrochemical monomers. The main challenges in the economical and sustainable production of FDCA are the selection of suitable substrates, and the development of an efficient catalytic-solvent system for its conversion via the 5-hydroxymethylfurfural (HMF) route.
- To address these issues, this research project intends to replace high value sugars such as fructose and glucose with whey, a dairy industry byproduct. In the first step, we analyzed different concentrations of whey permeate for HMF production.
- Lactosucrose demand has been on the rise due to its prebiotic and technofunctional properties. The most effective biocatalysts for targeted bioconversion of lactose into lactosucrose were identified. Our biocatalytic process has led to more than 87% wt% conversion of lactose in whey permeate to lactosucrose.
- Lactobionic acid is a high value-added lactose derivative which has recently emerged as a promising substance with countless applications in the food, pharmaceutical, cosmetic, medicine, and chemical industries. Different biocatalytic systems were developed for a complete conversion of lactose in whey permeate and milk permeate into lactobionic acid.

Objectives

Development of the scientific and technological basis of new approaches to enhance carbohydrate-rich by-products (lactose, whey) into high-value products and contribute to the sustainability of the dairy industry. Two main approaches are investigated:

A) Conversion of lactose whey into value added fine chemicals (HMF, FDCA, FDCA-aliphatic acid co-polyesters) through chemical conversion pathways.

B) Biotransformation of lactose whey into added value ingredients, Lactosucrose and Lactobionic acid, using enzymatic processes.

Results and potential benefits

This research activity is part of an overall approach for the recovery of food processing residues to protect the environment and preserve our resources. It is known that the worldwide whey production is estimated at 180-190 x 106 ton/year, out of which only 50% is further processed. The dairy industry is currently dealing with the whey permeate surplus by selling it as dry permeate powder, through incorporation into animal feed, or simply disposing it as waste. However, the disposal of surplus whey requires extensive pretreatments due to its high biological oxygen demand (30 to 50 g/L), which incur additional operating costs to the dairy industries. The dairy industry by-products represent tremendous potential as they can be converted into furanic compounds or high value-added ingredients.

Results and potential benefits, suite

Synthesis of biobased 2,5-furandicarboxylic acid (FDCA) monomers and their corresponding copolyesters for food packaging

- Lactose was studied as the model carbohydrate, which afforded 59 mol% HMF yield at the optimum conditions (130 °C, 100 min, and 0.21 mol/L AlCl3·6H2O). With whey permeate powder, 74 mol% HMF yield was obtained in a shorter reaction time (40 min) with the sample weight. The increase in HMF yield for whey permeate powder was attributed to the free amino groups of the proteins present, which concomitantly affected the reaction mechanism for HMF synthesis.
- The next challenge is the development of a base free catalytic system to oxidize HMF to FDCA. Therefore, we have developed a Mn-Fe3O4 magnetically recyclable catalysts for the oxidation of HMF to FDCA. To date, we have achieved 40 wt% FDCA yield and 100 wt% HMF conversion. The effect of different base, oxidizing agent, reaction temperature and time will be analyzed to further improve the yield.

Development of economically viable biotransformation technologies that would produce high value-added natural health sweeteners and functional ingredients

The results contribute to the development of the fundamental and technological bases of the targeted processes. Indeed, the most effective biocatalysts or biocatalytic systems for targeted bioconversions were determined. In addition, the important reaction parameters to be controlled and modulated, for a viable yield, were identified. Assessing the limits of these technologies according to their industrial potential will be part of our efforts.

The structural characterization of the biogenerated functional ingredient properties will make it possible to determine their potentials while highlighting their distinctive benefits on the basis of their structures.

Innovative aspects

- The study finds the efficient catalytic system which can transform whey permeate to HMF and FDCA.
- We have developed three different magnetic catalysts for HMF to FDCA oxidation.
- We are also building a one pot reactor where azeotropic mixtures will be used for Whey to HMF conversion.
- We have found out that amino acids play a catalytic role in HMF yield enhancement.
- The most efficient biotransformations for the conversion of whey permeate and milk permeate into lactosucrose and lactobionic acid were determined.

Professionals trained

- **Surabhi Pandey** (Ph.D. Candidate)
  - Surabhi wants to become a university professor. Her interests are between food and chemical engineering, where she is interested in developing practical applications (packaging) for food residues.
  - She is currently learning about green chemistry, catalysis, and polymerization.
- **Elham Chidar** (MSc)
  - Elham completed her experiments (lactobionic production) and she is currently writing her MSc thesis
- **Rami Bahlawan** (MSc)
  - Rami is working on the last part of his MSC that aims at the immobilization of biocatalysts on solid supports to increase their efficiency and reusability.
- **Dr Eugenio Spadoni** (postdoc)

For further information

In the first year, we wrote a review paper titled "Untapped potential of 2,5-furandicarboxylic acid and its copolyesters for food and beverage packaging". This manuscript needs some minor changes and will be submitted soon. The first objective has been completed and has been submitted in Chemical Engineering Journal titled as "Catalytic conversion of whey permeate into 5-hydroxymethylfurfural in a green solvent system". Conferences and talks will be given at different meetings as the Novalait Forum Techno.

Financial contributions

RITA Recherche Innovation Transformation Alimentaire: a consortium mandated by MAPAQ at McGill University in collaboration with CTAQ and MEI. Below is the total budget for the 13 activities of the RITA network.

Total budget: $2,765,828

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An eco-efficient approach to valorizing buttermilk

Duration: 2019-2022

Highlights

- Buttermilk is a co-product of butter production obtained by churning cream, a process that breaks down the milk fat globule membrane (MFGM).
- While butter consumption is on the rise in Quebec, buttermilk remains an untapped resource despite its potential as a high added value bio-ingredient, specifically when derived from the milk fat globule membrane.
- However, the milk fat membrane fragments contain molecules that make processing buttermilk into cheese difficult. Separating the membrane components would make it possible to valorize the non-fat solids in different dairy matrices to improve its eco-efficiency.
- The objective is to develop different strategies to separate the MFGM from buttermilk by combining ultra high-pressure homogenization (UHPH), a continuous process that promotes protein aggregation, with baromembrane processes.
- The project aims to optimize the use of buttermilk by valorizing its non-fat solids and deriving high added value bio-ingredients from the MFGM.

Objectives

The main objective of this project is to develop eco-efficient separation strategies to optimize the use of buttermilk in the production of MFGM-rich, high added value bio-ingredients and to valorize buttermilk's non-fat solids in dairy matrices.

More specifically, the objectives are to:

1) Characterize the impact of the UHPH process parameters on the size of buttermilk components and determine the impact of concentrating buttermilk through reverse osmosis on the size modifications brought about by UHPH;
2) Optimize microfiltration (MF) operating parameters to maximize filtration selectivity and efficiency during the fractionation of buttermilk's MFGM processed through UHPH;
3) Determine the impact of incorporating buttermilk proteins that have been defatted through the UHPH/MF process in dairy matrices.

Results and potential benefits

This project will provide conclusive data on the separation of the MFGM from buttermilk. UHPH may have a major impact on membrane selectivity/permeability during MF separation by modulating the size of the components in buttermilk, specifically casein micelles and MFGM fragments. In addition, the effect of MF parameters (pressure, velocity and recirculation speed) on fractionation yield and selectivity, as well as on membrane fouling, will generate unique theoretical and technological knowledge about the fractionation of buttermilk processed through UHPH to recuperate the MFGM. The ability to produce MFGM concentrates will allow for the development of new added value applications for buttermilk. This will create opportunities to increase revenues for Quebec's dairy industry while restoring the technical-functional properties of buttermilk proteins and valorizing buttermilk's solids in more conventional applications, such as yogurt and cheese production. In the end, the project will lead to a better understanding of the denaturation and protein interaction caused by UHPH and the development of innovative new approaches to separate the MFGM from buttermilk.

Innovative aspects

- Total recovery of buttermilk components in two fractions: the bioactive components of the milk fat globule membrane (MFGM) and the techno-functional components of the buttermilk.
- Innovative combination of ultra-high pressure homogenization and baromembrane filtration to separate the MFGM from other buttermilk components.

Professional trained

- **Serine Touhami**, master's student

For further information

Research results will be promptly transferable to the dairy industry through different channels, including the STELA Colloquium, Novalait's Forum Techno and other scientific conferences. Other communication activities, including articles and presentations, will be planned for collaborating users.

Financial contributions

Special call for projects: Dairy production and processing (2016–2021):

- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Quebec consortium for industrial bioprocess research and innovation (CRIBIQ)
- Novalait

Total budget: $167,337

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Improving dairy processing eco-efficiency using an integrated approach to dairy fluid concentration

Duration: 2019-2022

Highlights

- Milk contains more than 88% water. Milk composition does not change significantly when used to produce drinking milk (other than as a result of the standardization of its fat content) as opposed to milk used for cheesemaking where 90% of its volume is found in whey.
- Filtering milk to extract water and concentrate proteins is a widespread practice in dairy technology. It makes the production of a host of foods possible, in addition to making processing more eco-efficient. To do so, "baromembrane" processes are used, including reverse osmosis and ultrafiltration, which is garnering a growing amount of interest. The result is a concentrate that is rich in proteins, and the creation of whey, a liquid by-product.
- The residue from reverse osmosis presents a composition that is very close to water and can be used in dairy plants to reduce the consumption of potable water, whereas the whey obtained through ultrafiltration contains lactose and mineral salts that make it difficult to use.
- The idea of pre-concentrating dairy fluids (RO) to reduce transportation costs and the environmental impact (GHG) is attracting attention a context of eco-efficiency (EE).
- This project will measure the energy gains obtained by applying these new practices to cheese processing and the valorization of whey in an industrial context. It will draw on the knowledge and simulation software recently developed by the NSERC-Novalait Industrial Research Chair on Process Efficiency in Dairy Technology.
- The results will then be used to complete an initial evaluation of the potential of using whey obtained through ultrafiltration on dairy farms.

Objectives

The main objective of this project is to develop knowledge that will allow dairy processors to integrate the use of dairy concentrates obtained through RO or UF.

More specifically, the objectives are to:

1) Optimize cheese production conditions using dairy concentrates obtained through RO and UF;
2) Quantify the eco-efficiency gains obtained through the generation of cow water through RO in cheese-making;
3) Optimize the use of concentrates to improve material flow in the dairy processing industry.

Results and potential benefits

Objective 1: **New approaches** to correcting production defects resulting from the **use of reverse osmosis concentrates in cheesemaking** are being developed.

- The **addition of sodium to a reverse osmosis concentrate** shifts the mineral balance to milk's soluble phase and demineralizes the casein micelle.
- The work in progress aims to determine how this approach **improves the cheesemaking performance** of reverse osmosis concentrates.

Objective 2: A first **water recovery simulation (Chamberland et al, 2020)[a] on the industrial scale for a plant processing 1000 m³ of milk/day into milk protein concentrates (MPC-56)** demonstrated that:

- the pre-concentration of skim milk through RO prior to UF **reduced water and electricity consumption by 35% and 10%, respectively**, compared to the traditional process; and
- reductions in water and electricity consumption directly resulted in a **smaller environmental footprint of the MPC-56 production process** which improves eco-efficiency.

A simulation on the **impact of concentration through membrane processes in an industrial cheese plant** was completed. (Benoit et al, 2020)[b]

Objective 3: The research results will also **generate preliminary data** on the **impact of preconcentration of whey through RO** prior to transport. The simulations will help determine the scale (volume of whey), the volume concentration factor (VCF), and the transport distance (km) where pre-transport concentration becomes economically viable.

The project will produce significant benefits with respect to the use of milk components (including water) and reducing the environmental footprint of dairy transportation and milk processing processes.

Innovative aspects

- New approaches to correcting production defects resulting from the use of reverse osmosis concentrates in cheesemaking.
- First simulations:
  - water recovery simulation on the industrial scale for a plant processing milk into milk protein concentrates.
  - impact of integrating concentration through membrane processes on cheese plants' eco-efficiency
  - economic and environmental benefits related to the pre-concentration of whey prior to transport.

Professionals trained

- **Marie-Pier B. Vigneux**, PhD candidate.
- **Scott Benoit**, Postdoctoral fellow, Université Laval

For further information

The research results will be able to be quickly transferred to the dairy industry through diverse means, including the STELA Colloquium, the Novalait Forum Techno and different scientific conferences. Other communications activities (articles and presentations) are planned for collaborating users.

Financial contributions

Special call for proposals in dairy production and processing (2016–2021):

- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Quebec consortium for industrial bioprocess research and innovation (CRIBIQ)
- Novalait

Total budget: $177,996

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a. Chamberland, J. Bouyer, A., Provault, C., Bérubé, A., Doyen, A., Pouliot, Y. (2020) Efficiency assessment of water reclamation processes by reverse osmosis in milk protein concentrate manufacturing plants: a predictive analysis. J. Food Eng. 272:109811.

b. Benoit, S., Chamberland, J., Doyen, A., Margni, M., Bouchard, C., Pouliot, Y. (2020) Integrating Pressure-Driven Membrane Separation Processes to Improve Eco-Efficiency in Cheese Manufacture: A Preliminary Case Study. Membranes, 10, 287; doi:10.3390/membranes10100287

Characterizing the structure of stirred yogurt through digital technology and the mapping of critical production points

Duration: 2020-2022

Highlights

- In 2018, 387,707 tonnes of yogurt were produced in Canada, of which more than 75% were produced in Quebec. Canadians consume mostly stirred yogurt.
- The pace of development of new products is very fast in the yogurt industry and manufacturers need to provide advanced formulations to meet consumer expectations.
- Stirred yogurt is obtained by breaking the gel after fermenting in tanks.
- The structure of stirred yogurt can be summarized as a microgel suspension (gel fragments) interconnected in serum. The characterization of these microgels could predict certain structural properties, such as viscosity, firmness, and syneresis.
- Recently, a fast and inexpensive microscopic image analysis method was developed in an experimental context.
- This project aims to validate the use of this method with a range of products representative of the diversity of products on the market.
- A generalized mapping document for use by yogurt manufacturers will provide an overview of the critical points in the production process for stirred yogurt.

Objectives

The objectives of the project are to:

- Map the determinants of the structural properties of stirred yogurt;
- Summarize the mapping in a simple, easy-to-read document for quick decision-making;
- Validate a simple and fast method of characterizing the structure of stirred yogurt by surface analysis using digital imaging.
- Provide two new tools for yogurt manufacturers to support their product improvement and innovation efforts.

Results and potential benefits

Making yogurt is a complex process with numerous parameters that must be controlled and optimized to obtain a product that consumers enjoy. Among the quality criteria is creaminess, which is defined as a thick product (viscous, firm) with a smooth (lump-free) texture and a homogeneous appearance (no serum separation). The digital image analysis method provides a quick overview of the structure of stirred yogurt based on microgel size and heterogeneity. A correlation between the product's microstructure and the physical properties of viscosity, firmness, and syneresis was demonstrated in a previous experimental yogurt project using this method. This project aims to evaluate the predictive accuracy of this method on eight different commercial yogurts (varying in fat content and the presence of stabilizers). These yogurts have been generously provided by Quebec industrial partners. Early results suggest a link between the structural characteristics and the properties under study. This new method would provide stirred yogurt manufacturers with a new, fast, and inexpensive quality control and R&D tool.

A previous research project identified and studied specific critical points during the stirring and smoothing process for stirred yogurts. This project was carried out on a technical pilot scale 30 L of production) by testing various combinations of processes and formulations. A mapping document will summarize in a clear and concise manner all the results obtained from this project, as well as the key research data from other scientific teams, providing an up-to-date synthesis of knowledge on the subject. The purpose of this document is to be able to provide information as quickly and simply as possible to help manufacturers make quick decisions based on data from several years of scientific research.

Innovative aspects

- Simple and fast method validated on commercial products.
- Use of digital technology to characterize the structure of stirred yogurts.
- Distribution of a summary document to help manufacturers make quick decisions about formulations and stirring processes.

Professionals trained

A research professional, Ms. **Audrey Gilbert**, is working full-time on the project.

For further information

The research results will be promptly transferable to dairy farmers. Training video capsules will be produced to demonstrate the imaging technique. The mapping will be available on the Novalait website in addition to being distributed to dairy manufacturers. Other communication activities (articles and presentations) are planned for collaborating users (Novalait, Lactanet, etc.).

Financial contributions

- TransformAction: An initiative supported by an agreement between the Ministère de l'Économie et de l'Innovation (MEI), the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ), and the Conseil de la transformation alimentaire du Québec (CTAQ)
- Novalait

Total budget: $134,818

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Educational leadership chair in cheese technology

Duration: 2020-2025

Highlights

- The cheese industry is facing a growing shortage of skilled labour at both the artisanal and industrial scales.
- Creating an Educational Leadership Chair (ELC) in Cheese Technology is one way to address labour needs.
- The chair will establish a university-level training program, in addition to developing a research program exclusively dedicated to cheese-making technologies.
- ELC graduates will be better prepared for the realities of the industry. They will be able to support innovation to ensure that Quebec's cheese industry remains competitive in this new context of free trade, in addition to integrating sustainable development concepts directly applicable to cheese production.
- The proposed training program includes the creation of new cheese technology courses for Université Laval's food science programs as well as continuing education opportunities such as a spring semester school (*École du printemps*) for actors in the cheese industry associated with the production sector.
- Research activities will focus on enhancing control over cheese variability and improving the efficiency of manufacturing processes.

Objectives

- To create an internationally recognized centre of excellence in cheese production;
- To develop a university-level training program in cheese production;
- To bring industry stakeholders together around new continuing education projects;
- To implement a research program exclusively dedicated to cheese-making and that addresses industry needs;
- To generate new knowledge in order to boost industry competitiveness.

Results and potential benefits

- **Economic benefits**
  - **Enhanced industry competitiveness:** The ELC aims to provide multi-disciplinary training and develop new knowledge to improve the industry's competitiveness.
  - **Reduced product variability:** Determining the sources of cheese variability is at the heart of the ELC's research program. Further characterization of milk is envisaged to develop the milk standardization strategies of tomorrow.

- **Environmental benefits**
  - **Improved dairy plant efficiency:** Through the research program, the chair will focus on comparing technological itineraries for a reduced impact on resource use, as well as on developing new technologies that allow for better use of "overlooked" dairy components such as lactose.

- **Social benefits**
  - **Better understanding of citizen and consumer expectations:** Combining concepts in cheese-making technologies and industrial performance management with business internships, the training program will better prepare food science graduates for the realities of the industry and allow them to better meet consumers' increasingly high expectations.

Innovative aspects

- Creation of a university-level training program in cheese-making that alternates between classwork and business internships (4 months).
- Development of management skills to fine-tune student training in cheese-making technologies.
- Continuing education: creation of a spring semester school focused on adapting cheese-making processes.
- Development of a research program focused on the needs of the cheese-making sector.

Professionals trained

The first graduates of the training program will enter the labour market by 2025. In terms of research, the ELC's budget allows for the recruitment of one undergraduate student, three master's students, and one PhD student for the five-year duration of the chair.

For further information

The chair's work have began in 2020. Novalait's Forum Techno 2023 will be an opportunity to present the first research results, which will also be published in peer-reviewed scientific journals, in addition to being presented at national and international conferences. Further, articles aimed at the general public will be written to raise awareness of the chair and its activities.

Financial contributions

The chair is also funded by cash and in-kind contributions from:

- Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ)
- Novalait
- Agropur Dairy Cooperative
- Lactalis Canada
- Saputo
- Conseil des industriels laitiers du Québec (CILQ)
- Centre d'expertise fromagère du Québec (CEFQ)

Total budget: $937,750

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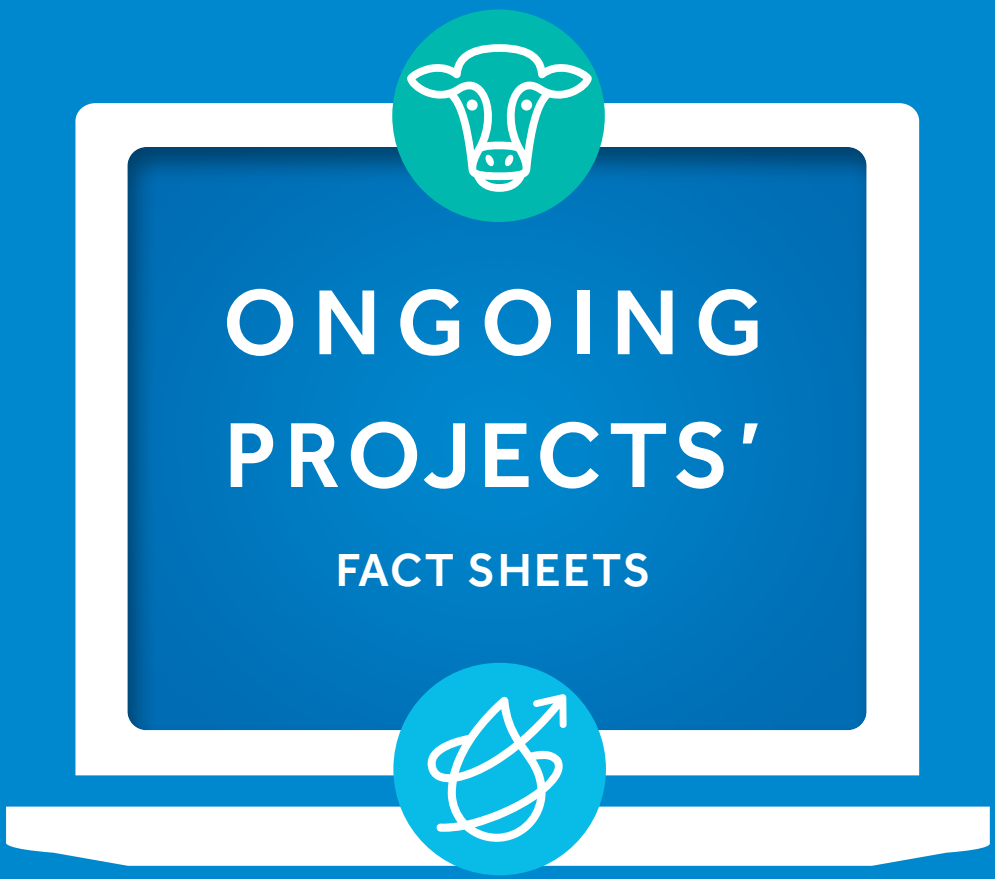

ONGOING PROJECTS'

FACT SHEETS

2021

Novalait

A milk sample to predict a dairy cow's health and welfare status?

Duration: 2016-2019

Highlights

- To date, no research carried out around the world has managed to develop a biomarker that would allow us to easily detect the health and welfare status of a dairy cow based on a simple milk sample.
- The rationale of the current research project is that:
  - Research on animal welfare has led to the development of reliable behavioural indicators, ranging from clinical signs to measurements of activity.
  - Blood metabolites such as NEFA and BHBA are sensitive indicators of specific health disorders, but currently no indicator has allowed one to quantify the general welfare status of animals. Moreover, the collecting and analysis of such blood indicators is often costly and technically demanding, on top of being stressful for the animals.
- The objective of this project is to develop precise indicators of dairy cow health and welfare that could be measured routinely in inexpensive and non-invasive milk samples from individual cows or from herds.
- In addition to the behavioural data collected during the experiments led by the Chair on the Sustainable Life of Dairy Cattle, physiological indicators were analyzed. These analyses identified different relationships between physiological and behavioural measurements of welfare and validated the usefulness of this new method of detecting the welfare status of dairy cows.

Objectives

- Developing precise biomarkers that can be recorded routinely (for example, through samples collected for milk recording) and used to detect herds and cows with a lower health and welfare level.

Results and potential benefits

This project integrates with the work being completed by the Research Chair on the Sustainable Life of Dairy Cattle , As part of the first theme of "Cow comfort and herd management," **physiological measurements were added to behavioural measurements**.

Over the course of 36 months, **four experiments** were conducted on the Macdonald Campus Farm of McGill University. These experiments aimed to evaluate the impact of stall configuration on the comfort and movement opportunities of tie-stall dairy cows. Four aspects were evaluated:
1. position of the tie-rail; 2. chain length; 3. stall width; 4. stall length-manger wall height combination.

Behavioural measurements were collected to evaluate the cows' ease of movement and activity. Thanks to various technologies, we can automatically collect certain data, which aids in the detection of welfare issues in tie-stall barns:

- The number and quality of lying and rising bouts, total lying time, number of steps, use of available space, etc.

Milk samples and blood samples were collected 3x/week during the first two weeks of each experiment, and 1x/week for the following 4-8 weeks. Physiological measurements were collected according to the following four secondary objectives:

1. **Identify and evaluate the link between various indicators of health and welfare**:
   - plasma, milk and behaviour indicators;
2. **Establish indicators allowing to effectively discriminate between cows with low and high levels of welfare**;
   - Welfare level = comfort level (ranging from low to high) provided to lactating cows in their tie-stalls;
3. **Define and refine the use of these indicators (alone or in combination) on the basis of cow and environmental indicators, including**:
   - Improved comfort level, stage of lactation, number of parities;
4. **Evaluate the potential to detect changes in the welfare status using the same type of spectroscopy as what is currently used for milk recording**:
   - FTIR spectroscopy.

Results and potential benefits

The work and data analysis identified links between housing changes and different biomarkers analyzed by FTIR spectroscopy. These results showed that it is possible to use regularly collected milk samples to identify cows with low or high welfare levels (Bahadi et al., 2021 Foods 10(2):450). More work is needed to further refine the method to more accurately identify the impacts of specific modifications on cow welfare status.

- This is a completely new method whose effectiveness has just been demonstrated.
- This method is ground-breaking because it allows for the remote monitoring of the welfare of dairy cows in a non-invasive and inexpensive way.

Professional trained

Behavioral Measurement Component: - 4 thesis-based master's students
Sarah McPherson
Véronique Boyer
Jessica St. John

The work of these students was carried out as a part of the Chair on the Sustainable Life of Dairy Cattle, which will be completed in collaboration with the following two students:

Physiological Measurement Component: - 2 students
Audrey St-Yves, M.Sc. student
Mazen Bahadi, Ph.D. student

Daniel Warner (Postdoctoral fellow)

For further information

- An article featuring the project can be found in the June 2017 edition of the magazine *Le producteur de lait québécois*
- Project supervisor's website: mcgill.ca/animal
- Blog: cowlifemcgill.com
- Twitter account: @CowLifeMcGill
- Student presentation: Mazen Bahadi at the 2021 Novalait Forum Techno
- Bahadi M., Ismail A.A., and E. Vasseur. 2021. Fourier Transformed Infrared Spectroscopy as a Tool to Study Milk Composition Changes in Dairy Cows Attributed to Housing Modifications to Improve Animal Welfare. Foods 10 (2):450.

Financial contributions

Partnership research program of the FRQNT (Fonds de Recherche Nature et Technologies du Québec), in collaboration with:

- The Québec consortium for the industrial bioprocess research and innovation (CRIBIQ)
- Novalait
- Valacta

Collaborative Research and Development (CRD) Grants from Natural Sciences and Engineering Research Council (NSERC).

Total budget: $261,404

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Improving cows' protein diet through new models tested in Quebec

Duration: 2018-2021

Highlights

- To ensure their economic, environmental and social sustainability, dairy farms in Quebec must reduce their environmental footprint while remaining profitable.
- A number of models are used in different countries to evaluate the protein supply in rations to balance them with the needs of high-yielding dairy cows in order to reduce production costs and nitrogen dejections without compromising yield.
- Nitrogen (N) excretion is a major problem: on average, 30% of nitrogen is recuperated in milk proteins, with fecal and urinary losses representing 35% and 35%, respectively, of the nitrogen ingested.
- The goal of this research is to validate the impact of a revision of three cow ration formulation models on protein and amino acid recommendations in the Quebec context.
- Two of the most popular models in North America will be compared–the 2001 National Research Council (NRC) model and the 2018 revision and the Cornell Net Carbohydrate and Protein System (CNCPS v.6.5, 2015), the NorFor 2011 Scandinavian system as well as the new French system, INRA (2018).

Objectives

The goal of this research is to validate the impact of a revision of three cow ration formulation models on protein and amino acid recommendations in the Quebec context.

More specifically, the objectives are to:

1) Determine the influence of ration characteristics on the models' capacities to predict protein supply by comparing the predictions from the three models with the reference model (NRC, 2001);
2) Compare the milk yields and milk proteins predicted by the four models with values reported in the literature and measurements from 100 farms in Quebec;
3) Propose adaptations to the ration formulation model used by the Quebec-Atlantic Centre of Expertise (Lactanet: currently based on NRC, 2001) and to the other models used in Quebec so that they reflect the particularities of rations used in Quebec.

Results and potential benefits

The proposed study will verify if the predictions from the new models also apply to the rations commonly used in Quebec, as opposed to those with an alfalfa/corn base (United States) or multiple types of feed (Europe). The project presents a unique advantage by comparing the predicted values to the values actually observed on Quebec dairy farms. If it is found that biases are present with regard to certain types of rations frequently used in Quebec, corrections to these biases may be integrated to better adapt the models used by Lactanet, and other organizations in Quebec, to the Quebec context. As a result, our study will validate and quickly transfer these advances to Quebec dairy farms, resulting in the application of the new American and European dietary models. These modifications will result in reductions in the amount of protein in rations, production costs and nitrogen emissions into the environment. The project will position Quebec as an innovative industry leader with regard to protein and amino acid (AA) recommendations for dairy rations.

Innovative aspects

- Comparison of model-predicted values to those actually observed on Quebec dairy farms.
- Positioning of Quebec industry at the fore front in terms of protein recommendations.

Professional trained

Simon Binggeli, PhD student.

For further information

The research results will be transferable to dairy farmers by way of the dietitians and experts who formulate the rations. An article will be written for the journal *Le producteur de lait québécois*. In addition, a presentation proposal will be submitted to CRAAQ for the Quebec Dairy Cattle Symposium. Other communications activities (articles, training sessions and presentations) are planned for collaborating users, including Novalait and Lactanet.

Financial contributions

Special call for proposals in dairy production and processing (2016–2021):

- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Quebec consortium for industrial bioprocess research and innovation (CRIBIQ)
- Novalait

Total budget: $177,996

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Performance and behaviour of dairy heifers according to their milk diet

Duration: 2018-2022

Highlights

- The milk feeding phase can affect growth and production parameters in heifers.
- Little information exists on the impact of modifying milk feeding of calves between the current Canadian recommendation and unrestricted feeding.
- Using a tape measure to record thoracic circumference is the tool most frequently used on farms to assess weight. This project helped validate that this technique works well for pre-weaned calves.
- Epigenetics sheds light on gene regulation to increase our knowledge of the impact of calves' consumption on this mechanism with respect to weight gain and future milk production.

Objectives

The objectives of this project are:

1) to evaluate the effect of different milk feeding management scenarios on weight gain in heifers (pre-weaning performance), cow productivity (post-weaning performance) and gene methylation (epigenetics);
2) to evaluate the impact of different milk feeding management scenarios on calf behaviour;
3) to validate the use of a tape measure to assess the weight of very young heifers.

Results and potential benefits

To attain these objectives, one experiment involving more than 300 heifers is currently underway at two dairy farms implementing a milk feeding phase with an automated calf feeder. The experiment has two different milk feeding management scenarios (restricted vs. unrestricted) as treatments. Data collection up until weaning is complete. Data was collected three times per week for the first three weeks of life, followed by every two weeks until the calves were weaned. To date, more than 300 calves have been recruited for the project and over 5,000 weight and height data items have been recorded. The experiment is being continued on one farm to obtain the impacts of the treatments on post-weaning performance. The heifers were weighed and measured before their first insemination. Milk production during the first lactation on the same farm is in the course of being recorded. Additionally, a sub-sample of the data will be used for epigenetic analysis. The project's current findings confirm the validity of using a tape measure to evaluate the calves' weight prior to weaning and show that, despite minor differences, both treatments result in behaviours that are typical of calves at that age. Data on milk feeding programs (restricted vs. unrestricted) will be used as a basis for making recommendations to farmers. There is currently a lack of guidelines for the feeding of young heifers in Quebec.

Innovative aspects

- Establishment of guidelines for feeding young heifers in the context of Quebec dairy farm production.
- Validation of the tape measure as a means of measuring heifer growth in the Quebec context.

Professionals trained

- **Marwa Hasnaoui**, master's student, tape measure validation
- **Ousmane Magassa**, master's student, calf behaviour
- **Léonie Laflamme-Michaud**, master's student, relationship between the milk feeding phase, pre-weaning performance and gene expression
- **Jennifer Philion**, master's student, relationship between the milk feeding phase and post-weaning performance in heifers

For further information

The research results will be promptly transferable to dairy farmers. An article will be written for the journal *Le Producteur de Lait Québécois*. In addition, a presentation proposal will be submitted to CRAAQ for the Quebec Dairy Cattle Symposium (*Symposium des bovins laitiers*). Other communication activities (articles and presentations) are planned for collaborating users, including Novalait and Lactanet. Already published:

- *Bulletin des agriculteurs* – November 2019: "Mesurons les jeunes génisses laitières"
- *Le Producteur de lait Québécois* – April 2020: "Le ruban québécois pour estimer le poids des veaux"
- *Poster at the Quebec Dairy Cattle Symposium* – November 2019: "Validation et développement de méthodes pour le suivi de la croissance pré-sevrage des génisses laitières"
- *Posters at the Quebec Dairy Cattle Symposium* – November 2020: "Comparaison du comportement des veaux laitiers Holstein nourris selon la recommandation canadienne actuelle et à volonté" and "Alimentation lactée à volonté ou selon la recommandation canadienne: impacts sur la croissance des génisses"
- *Poster at the American Dairy Science Association conference* – June 2019: "Use of body measurements to estimate live weight of Holstein dairy calves in the pre-weaning period"
- *Presentation summary at the Science Information Day – Dairy cows and fodder plants* – February 2019: "Validation et développement de méthodes pour le suivi de la croissance pré-sevrage des génisses laitières"

Financial contributions

Special call for proposals in dairy production and processing (2016–2021):

- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Quebec consortium for industrial bioprocess research and innovation (CRIBIQ)
- Novalait
- Ferme M.G. L'Heureux

Total budget: $255,035

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Developing diet strategies to improve protein efficiency on Quebec dairy farms

Duration: 2019–2022

Highlights

- Protein is the most expensive ingredient in rations fed to dairy cows. It accounts for 42% of feed costs.
- However, more than 70% of this investment is excreted by the animals without ever contributing to milk production.
- Dejections contain a large quantity of nitrogen, the base component of the amino acids that form proteins. A ration offering a better balance of amino acids would reduce nitrogen ingestion, consequently lowering feed costs without negatively affecting milk yield.
- Based on research developed by Agriculture and Agri-Food Canada (AAFC) in Sherbrooke, this project aims to demonstrate that it may be profitable on commercial farms to feed cows a diet with a better balance of amino acids.
- After studying the practices in use on 12 commercial farms equipped with feeding robots, the research team will test an amino acid-optimized ration and compare it to the ration normally used on the farm (control ration).
- The results of this project could lead to the development of feed strategies best suited to the reality of dairy farms.
- This project will also serve to showcase technological innovation and encourage the dairy cattle sector to use innovative practices with regard to diet and herd management.

Objectives

The main objective of this project is to validate the use of feed rations with reduced nitrogen levels and balanced amino acids in commercial conditions.

More specifically, the objectives are to:

1) Develop a typology on 12 commercial farms to establish the test rations to be used on the farms based on current practices;
2) Test an amino acid-balanced ration on a group of cows on each of the project's farms.

Results and potential benefits

The twelve commercial farms (total of 1,000 cows; average of 75 cows per farm) were met with and completed a questionnaire to provide an overview of the farm's production system (herd management, animal characteristics, milk quality, facilities, human resources). The rations to be tested in the context of this project will be defined based on this analysis. On each farm, the cows will be divided into two groups. The first group will serve as the control group and will receive the farm's normal diet. The second group will receive a ration with a crude protein content reduced by 1.5%, adjusted for the total need of metabolizable proteins and balanced for amino acids by using lysine, methionine and histidine. This project will also serve to showcase technological innovation and encourage the dairy cattle sector to use innovative practices with regard to diet and herd management. The demonstration will lead to the formulation of rations and dietary supplements based on the cows' true protein needs. Farmers, consultants, nutritionists and students will all benefit from this research.

Innovative aspects

- Project completed with the diversity and constraints of commercial farms, which bodes well for a rapid adoption of the conclusive results.
- Potential to lower the food costs of herds and reduce the volume of waste emitted into the environment without compromising milk yields.

Professional trained

- One Master's student, **Frédérika Nadon**, was recruited for the project.

For further information

The research results will be promptly transferable to dairy farmers. An article will be written for the journal *Le Producteur de Lait Québécois*. In addition, a presentation proposal will be submitted to CRAAQ for the Quebec Dairy Cattle Symposium (*Symposium des bovins laitiers*). Other communication activities (articles and presentations) are planned for collaborating users (Novalait, Lactanet, etc.). A presentation will also be given at Novalait's Forum Techno.

Financial contributions

Special call for proposals in dairy production and processing (2016–2021):

- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Quebec consortium for industrial bioprocess research and innovation (CRIBIQ)
- Novalait
- 12 dairy farms (8 in the Saguenay-Lac St-Jean region and 4 in the Quebec City region)

Total budget: $407,729

Contact persons

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Systems approach to deciphering molecular mechanisms of infertility in lactating dairy cows with subclinical ketosis

Duration: 2020-2023

Highlights

- Ketosis, especially subclinical ketosis is a very important metabolic disorder of lactating cows and it has significant negative impact on cow's health and reproductive performance.
- As reproductive failure is the major reason for culling of lactating cows from Canadian herds, understanding the mechanisms of ketosis and its impact on reproduction is important.
- This project aims to uncover the molecular mechanisms of subclinical ketosis and its impact on fertility in lactating Holstein cows.
- The results will help us develop therapeutic and management strategies to manage reproductive performance of lactating cows.
- We are working with data milk BHB and reproductive performance indicators from over 30,000 cows.

Objectives

We hypothesize that clinical and subclinical ketosis impact negatively on reproductive performance of lactating cows.

The objectives are:

- To analyze milk BHB data to categorize ketosis into multiple types based on the level and time of increase in milk BHB concentration.
- To analyze parameters indicative of reproductive performance in cows suffering from ketosis compared to health cows.

Results and potential benefits

In the last 15 months, we have been investigating the reproductive performance of cows with milk BHB levels indicative of clinical and subclinical ketosis using data from Lactanet and CDN of >30,000 cows. Ketosis was categorized into multiple types based on the levels and time of milk BHB increase. Our preliminary results reveal that prevalence of clinical and subclinical ketosis within the first 42 days of lactation was about 27%. As expected, Type 2 ketosis (occurring in first 2 weeks of lactation) was three times more prevalent than Type 1 ketosis (occurring in 3-6 weeks of lactation). The 6% prevalence of Type 1 ketosis is also important as it seems to affect about 1800 cows among the animals in this study. This demonstrates that cows need to be monitored for clinical and subclinical ketosis throughout the first six weeks of lactation. Within Type 2 ketosis, Type 2 subclinical ketosis is more prevalent than Type 2 clinical ketosis. This demonstrates the importance of monitoring subclinical ketosis using BHB levels in milk.

We are now analyzing the impact of individual types of ketosis on reproductive performance of the affected cows compared to fertility of healthy cows. The parameters to be analyzed are: 1) calving to first service interval, defined as number of days between calving and first service; 2) first service to conception, defined as number of days between first service after calving and conception; 3) days open, defined as the number of days between calving and service that resulted in a conception; and 4) calving interval, defined as number of days between two consecutive calving.

Innovative aspects

- Systematic categorization of ketosis types to investigate their impact on reproductive performance of lactating cows.
- The data of milk BHB levels and reproductive performance indicators are obtained from Lactanet database involving data from over 30 000 cows.

Professional trained

- Mr. **Teshome Alemu** (PhD student) – data analytics and bovine reproduction – this student received a scholarship for the first three years his doctoral studies.
- Dr. **Ejimedo Madogwe** – expert in bioinformatics and big data analytics. She recieved a postdoctoral fellowship for 2021-22 and is being partially funded by this project.

For further information

This is an ongoing project. We will publish the results at international conferences (e.g. Canadian Society for Animal Science) and research article in reputed journals (e.g. J of Dairy Science or Theriogenology). Moreover, some conferences will be given to different organisation as example The Novalait Forum Techno.

Financial contributions

Special call for proposals in dairy production and processing (2016–2021):

- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Quebec consortium for industrial bioprocess research and innovation (CRIBIQ)
- Novalait

Total budget: $343,645

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Development, evaluation, validation and implementation of FTIR-based technology for rapid microbial identification of pathogens associated with cow mastitis

Duration: 2020-2022

Highlights

- Rapid and cost-effective methods of identifying microbial pathogens can have a tremendous impact on milk quality and reduce losses due to quality defects associated with bovine mastitis.
- Current methods require the use of well-trained technicians, reagents, expensive instrumentation (e.g., MALDI TOF MS) and/or expensive genotyping (whole genome sequence or PCR) methods.
- We have developed a single-step technology [based on Fourier transform infrared (FTIR) spectroscopy] for the identification of microorganisms that is reagent-free and provides results in 1-2 minutes subsequent to initial microbial culture.
- This technology can be implemented on portable instruments suitable for on-farm use.
- For proof-of-concept, we are developing FTIR spectral databases for use in the identification of isolates from the Mastitis Pathogens Culture Collection maintained by the Réseau mammite/Mastitis Network at the Faculté de médecine vétérinaire de l'Université de Montréal. We have carried out an evaluation study of the method to demonstrate the successful discrimination (>99%) between Gram-positive and Gram-negative mastitis pathogens and between coagulase-negative staphylococci (CoNS) and S. aureus, and we have also achieved successful discrimination (>99%) between multiple species within the CoNS group.
- The next steps include integration of the methodology with commercially available on-farm culture kits and validation of the database for identification of bacteria isolated from the milk of Quebec dairy cows exhibiting subclinical mastitis.

Objectives

Our general objective is to develop cost-effective methodology for rapid species identification of bacteria, yeasts, and molds that can be implemented in the agri-food sector for on-site use by addressing the following questions:

- Can we develop a one-step method for microbial identification down to the species level?
- Can such a technology be used to discriminate between microorganisms implicated in cow mastitis and milk quality?
- Can the proposed technology provide comparable accuracy to current technologies?
- Can on-farm implementation be successfully achieved?

Results and potential benefits

Economic:
A one-step microbial identification technology capable of providing timely identification of contagious and environmental mastitis pathogens isolated from milk cultures will result in Improved mastitis prevention and control on dairy farms, leading to:

- Increased overall profitability of dairy farms
- Improved milk quality; reduction in product losses due to quality defects
- Increased productivity of the dairy sector

Environmental - For information only:

- Reduction of routine antibiotic use in dairy cows for prevention and control of mastitis (blanket dry-cow therapy)

Social - For information only:

- Improvement of animal health and welfare on Quebec dairy farms

Innovative aspects

- Simpler technology currently under development in our laboratory to identify species of bacteria, yeasts and molds.
- Focus for the first time on the identification of bacterial species isolated by bovine milk culture and associated with clinical mastitis or with subclinical mastitis resulting in unacceptable milk quality.
- Farmers and milk processors can benefit from increased low-cost surveillance of their animals and dairy-based products.

Professionals trained

- Ms. **Xin Di Zhu,** doctoral student (in progress), aims to develop expertise in addressing food safety issues, particularly in relation to microbial hazards, and is involved in collaborative projects in this area of research with labs at Health Canada and the Canadian Food Inspection Agency. In the present project, Ms. Zhu's roles are:
  - Developed FTIR spectral databases encompassing over 300 isolates obtained from the Mastitis Pathogens Culture Collection
  - Developed FTIR-based classification models for microbial discrimination and identification based on differences between the infrared spectra of the microorganisms
  - Tested the performance of the newly developed FTIR-based microbial identification methods against existing methods
- Ms. **Lisa Lam** and Ms. **Tamao Tsutsumi** (doctoral students)
  Both students are completing their doctoral theses concerning various applications of FTIR spectroscopy in clinical microbiology; they trained and assisted Ms. Zhu in all the aspects of her work listed above.

For further information

The results of this ongoing research project will be included in the doctoral thesis of Ms. Zhu and will be published in a peer-reviewed scientific journal such as Journal of Dairy Science. She will have the opportunity to present these findings to research participants at the Faculté de Médecine Vétérinaire de l'Université de Montréal and conferences will be given at different moment for example at the Novalait Forum Techno.

Financial contributions

RITA Recherche Innovation Transformation Alimentaire: a consortium mandated by MAPAQ at McGill University in collaboration with CTAQ and MEI. Below is the total budget for the 13 activities of the RITA network.

Total budget: $2,765,828

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Identifying bioprotective cultures that extend the shelf life of dairy products

Duration: 2019–2021

Highlights

- The shelf life of dairy products such as pasteurized milk, yogurt and grated cheese is limited because undesirable microorganisms eventually develop and create unpleasant odours and tastes.
- In North America, 20% of dairy products are lost or wasted, mostly by consumers at the household level.
- A promising strategy to stop the development of food-altering microorganisms is to control their growth using bioprotective cultures. Bioprotective cultures extend the shelf life of foods by producing natural antimicrobial compounds that slow the growth of undesirable microorganisms.
- The effectiveness of bioprotective cultures depends on the exchanges between microorganisms. This project will characterize the interactions between bioprotective cultures and alteration microorganisms by developing new, systematic methods on a large scale.
- This biopreservation strategy aims to develop mixes of bioprotective cultures for specific dairy products to extend their shelf life and help reduce food waste.
- The development of a consortium requires a prior understanding of the compatibility of strains since the properties of the mix are not equal to the sum of its individual parts. This phenomenon can be explained by the microbial interactions that take place within the dairy matrix and modify the behaviour of strains.

Objectives

The main objective of this project is to characterize the interactions between bioprotective cultures and alteration microorganisms by developing new, systematic methods on a large scale.

More specifically, the objectives are to:

1) Develop high-throughput methods to screen microbial interactions in a solid culture media;
2) Map the interactions of reference strains and strains isolated from dairy products;
3) Develop a tool to select strain consortiums;
4) Validate the selection tool.

Results and potential benefits

Dairy farmers and processors must meet the quality standards required by consumers. These standards are very high when it comes to shelf life, particularly for yogurt and grated cheese. Unfortunately, dairy processors have no control over what happens to products once they arrive at the grocery store. The use of bioprotective cultures is a natural solution for processors to limit problems of this type by creating a complementary barrier without having to use chemical additives. Bioprotective strains can interact with starter cultures and the endogenous flora of the dairy matrix. To select compatible strains with optimal bioprotective activity, in vitro tests are necessary. This project therefore addresses the challenge of systematically developing new bioprotective consortiums at a low cost since developing bioprotective cultures with the current methods is expensive and labour-intensive. The high-throughput methods developed will make this process available to dairy processors. The project will also shed light on the behaviour of different lactic acid bacteria (LABs) in a community context. For example, we were able to measure the competitiveness or cooperation between these bacteria toward the endogenous microbiota of dairy products and other LABs. We observed that the social qualities of these bacteria vary from one strain to the next. For example, the strain *Lactococcus lactis cremoris* promotes the growth of endogenous isolates while reducing the growth rate of other LABs. Conversely, the strain *Lactobacillus pentosus* reduces the production of endogenous isolates to the benefit of LABs. In addition, our experimental design simulates the impact of an alteration of microbial diversity through the addition of a starter culture or bioprotective strain. Diversity, specifically uniformity in the proportions of different microorganisms, significantly influences the production and growth rate of the total community (including endogenous flora). This new understanding of the impact of different strains and the modulation of diversity on other microorganisms in the dairy environment may help optimize strategies for adding microbial cultures to feed.

Innovative aspect

- The methods developed within the high-throughput microbial interaction mapping platform can be used to characterize the performance of (new) strains in a community context and rapidly identify combinations demonstrating optimal technological or bioprotective activities (e.g. competitiveness, antifungal or anti-pseudomonas activity, etc).

Professional trained

One PhD student, **Amadou Ndiaye**, will begin in May 2019. He is developing an expertise in high throughput approaches, including mass data analysis. After graduating, he wanted to return to work in the food processing industry.

For further information

The research results will be able to be quickly transferred to the dairy industry through diverse means, including the STELA Colloquium, the Novalait Forum Techno and different scientific conferences. Results will also be shared with the METABIOLAC chair scientific committee. Other communications activities (articles and presentations) are planned for collaborating users.

Financial contributions

Special call for proposals in dairy production and processing (2016–2021):

- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Quebec consortium for industrial bioprocess research and innovation (CRIBIQ)
- Novalait

Total budget: $186,311

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Development of multidimensional approaches to identify and select multifunctional natural ingredients

Duration: 2019-2022

Highlights

- Although chemical synthesis processes have made it possible to obtain a wide variety of ingredients, the health risks associated with some have caused consumer avoidance. To this end, the food industry is increasingly confronted with consumer demand for "clean label" products, with no additives and artificial preservatives, with labels of ingredients easy to read and recognize while maintaining appropriate storage times and optimal textural and sensory properties.
- Efforts to develop multidimensional approaches for the identification and selection of multi-functional natural ingredients that are easy to incorporate into a wide range of products are needed to support the development of "clean label" food products.
- Considering the emerging market for "clean label" products worth billions of dollars, Quebec's agri-food sector could benefit greatly from the development of a multidimensional platform of multifunctional natural ingredients.
- A platform-based network integrating all the mappings using artificial intelligence tools with the possibility of identifying the multifunctionalities of ingredients and the optimal conditions will not just increase the efficiency of the application of selected natural ingredients but also the capacity to innovate.

Objectives

Development of a multi-dimensional platform containing integrated multifunctional maps for multi-sectors as a tool for identifying natural ingredients of interest and their optimal conditions of use whose purpose is to accelerate innovation. The specific objectives include:

- Design a database of natural food ingredients using diverse sources of information and establish the scientific basis for multifunctionalities of natural ingredients at different stages of maturity.
- Develop multidimensional mappings of natural ingredients, which link structural and physicochemical properties to techno-functional efficiencies.
- Develop an active dynamic platform that integrates all mappings as tools for identifying natural ingredients and their optimal application conditions.
- Assess the possibility of developing predictive In-silico models.

Results and potential benefits

A database of existing or emerging natural multifunctional ingredients of interest to participating industries will be developed. The results will be used to develop a multifunctional and multidimensional approach that will help to understand the synergy between the different techno-functionalities of natural ingredients. The use of multi-dimensional mapping focusing on the multifunctionality of ingredients will help to limit the number of ingredients that are needed to formulate selected "clean Label" food products. The application of artificial intelligence techniques will provide industry partners with a platform (in the form of a network) that links multidimensional mappings as fast tools of formulation engineering that will not only facilitate the identification of ingredients to substitute artificial ingredients, but also the discovery of new multifunctionalities of interest for the agri-food sectors involved.

Innovative aspects

- An innovative database of existing or emerging natural ingredients that can facilitate the identification and the selection of appropriate ingredients.
- Identification of multifunctionalities and their implementations as tools of formulation engineering "Genie de Formulation".
- Discovery of new applications of existing or new ingredients through the in-silico analyses.

Professionals trained

- **Nandini Kodomagge**, MSc non thesis
- **Diksha Rani**, MSc non-thesis
- **Nitin arora**, MSc non-thesis
- **Amalie Younes**, BSC Food Science
- **Kevin Misaiphon**, BSC Food Science
- **Muriel Yok Kam Wong Min**, BSC Food science

For further information

The successful completion of this project and the training of researchers will provide unique opportunities for the technology transfer. Other mean of transfer includes the periodic scientific meetings and discussion of the results with the various industrial partners of this research activity. Presentation of the results (oral and posters) to all members of the network at the meetings and workshops does also contribute to the transfer. A presentation at the Forum Techno will be planned. The publication of scientific articles and the presentation of the results in scientific meetings will also be other means of transfer.

Financial contributions

RITA Recherche Innovation Transformation Alimentaire: a consortium mandated by MAPAQ at McGill University in collaboration with CTAQ and MEI. Below is the total budget for the 13 activities of the RITA network.

Total budget: $2,765,828

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Development of active packaging solutions for the extended shelf life of sensitive food products

Duration: 2019–2022

Highlights

- The trend of natural foods without additives or preservatives has gained momentum in recent years. The rise of clean labelling requires food processors to make a special effort.
- A number of recent studies have demonstrated active packaging's potential in various uses related to shelf life. The release of antioxidants, gaseous compounds and antimicrobial agents and the absorption of specific molecules are just some examples of potential types of activation.
- The use of these technologies could limit food waste as well as contamination and poisoning risks in addition to prolonging the shelf life of packaged foods.
- However, releasing such packaging solutions to market is a sizable challenge in the food industry. The increased costs associated with these technologies remain an obstacle to widespread use.
- The potential benefits of this project for the food industry are considerable. Perishable and high value—added products could benefit from any active packaging technologies developed as part of this project. For example, fresh cuts of meat and fish could last longer because of the use of antibacterial film.

Objectives

- Develop active packaging solutions enabling the prolonged shelf life of sensitive food products
- Reduce food waste

Results and potential benefits

This project will focus on the development and testing of packaging materials (paper-, textile- or polymer-based or a mix) containing various functional components (e.g., antimicrobial, agents and antioxidants). The solutions developed will take into account legal constraints in terms of migration limits, the environment (through the use of biodegradable polymers) and commercialization potential (industrial feasibility and associated costs). This active packaging will act as an alternative to food additives resulting in shorter and healthier ingredient lists (clean labels).

- Economic aspects: Financial advantage for the adopting industry through the extended shelf life and freshness of fresh products
- Environmental aspects: Reduced food deterioration and food waste
- Social aspects: Reduced potential risks to food safety

Innovative aspects

- Development of flexible multi-layer packaging solutions containing “active” molecules
- Immobilization of active molecules (e.g., antioxidants, antimicrobial agents, drying agents and moisture-control agents) in different types of packaging materials (e.g., paper or polymer) and different controlled-release strategies
- Nanomaterials (metal and/or composites) incorporated in packaging materials with improved and sustainable features in terms of antimicrobial and antioxidant properties, freshness retention, and gas barriers.

Professionals trained

- **Satwik Majumder**, PhD student at McGill University, under the supervision of Dr. Saji George

  The expertise acquired is in animal handling, analytical equipment, microscopy, and data analysis. His research interests are sustainable agriculture and the environmental and food applications of nanotechnologies.

For further information

Knowledge transfer to industry partners: The results will be presented to corporate partners of the research project. There will be talks at Polytechnique. In addition, a talk may be given at Novalait’s Forum Techno. The students involved in the project will write articles.

Financial contributions

RITA Recherche Innovation Transformation Alimentaire: a consortium mandated by MAPAQ at McGill University in collaboration with CTAQ and MEI. Below is the total budget for the 13 activities of the RITA network.

Budget total : 2 765 828 $

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Development of antifungal ingredients leveraging antimicrobials from natural sources and an encapsulation process to maintain the quality of grated cheese

Duration: 2019-2022

Highlights

- Cheeses are an at-risk product with respect to contamination by undesirable moulds. To remedy this problem, natamycin is commonly used as an antifungal on the surface of whole and grated cheeses.
- However, consumers are increasingly concerned about the ingredients added to products, prompting companies to offer different alternatives that use natural antimicrobial extracts.
- It has been shown elsewhere in the world that a number of molecules from extracts of fruits, spices, plants, and algae have antifungal properties.
- Some Quebec algae extracts have antibacterial properties, but they are not documented.
- Several algae extracts have been produced (extracts of carrageenans, proteins, lectins, ethanolic extracts, terpenes) and their antifungal activity has been evaluated but no antifungal activity has been detected to date.
- Two arctic bacteria that show a broad spectrum of antifungal activity have also been isolated and the research team is attempting to identify the active antifungal molecule.
- Natural antifungal compounds of fruit and plant extracts have been selected and characterized and a formulation with synergistic extracts has been developed. Formulation stabilization studies are underway.

Objectives

The research hypothesis is that natural extracts (spices, fruits, algae, metabolites of arctic bacteria) contain antifungal compounds that can replace natamycin in grated cheese. To verify this hypothesis, the project has the following objectives:

- Prepare different natural extracts and test their antifungal activity;
- Purify and characterize antifungal molecules;
- Develop formulations containing synergistic compounds;
- Develop a method for stabilizing the formulations developed;
- Assess in situ the effectiveness of formulations to improve shelf life;
- Evaluate the sensory properties of coated cheeses;
- Assess the safety and toxicity of anti-fungal extracts derived from algae.

Results and potential benefits

The antifungal capacity of natural extracts (essential oils (EOs) and citrus extracts) against cheese-altering moulds has been determined and a formulation containing these synergistic extracts has been developed. Microfluidization of this formulation in the form of a nanoemulsion has been shown to be effective at ensuring its stability. The project will also reduce energy consumption and allow for the application of low-cost (physical) processes. The research team has also discovered two new antifungal molecules that are effective against a wide range of fungi. This may lead to the development of new products for the cheese industry.

The potential results and benefits of the project include the identification of a variety of new molecules from natural sources presenting antifungal properties in refined cheeses. Their effectiveness at inhibiting mould growth in grated cheese and extending its shelf life while maintaining its physicochemical and organoleptic quality may also be demonstrated.

Innovative aspects

- The characterization of natural extracts will explore several properties of spices, fruits, algae, and metabolites of arctic bacteria that have not been studied to date and may be of interest in food preservation.
- The use of encapsulation processes for natural bioactive compounds using a «clean label» approach is also an innovative approach to ensure the stability of the formulations developed and to optimize their bioactivity during processes and storage.

Professionals trained

- **Ghada Sassi**, Master's student in applied microbiology, INRS - Centre Armand-Frappier.
  - Expertise acquired: assessment of antimicrobial effectiveness; mastery of analytical processes and use of advanced technologies such as modelling, nanotechnology, encapsulation for the development of state-of-the-art technologies applied to food.
  - Professional interests: Research, design, and development of complex food formulations, elaboration of processes for the development of new technologies (etc.).
- **Adam Classen**, master's student, McGill University. Adam is responsible for co-culture experiments and is attempting to determine the molecular identity of new antifungal molecules.

For further information

Research results will be transferred to dairy industry partners at scientific meetings and communication activities such as the Novalait Forum Techno and the STELA Symposium. Results will also be presented at the annual conference of the Canadian Society of Microbiologists in 2021 and published in the following journals: *International Journal of Antimicrobial Agents*, *Int J. Food Microbiology*, *LWT Food Sc. Technol.* etc... These activities will be coordinated by the RITA Consortium management team.

Financial contributions

RITA Recherche Innovation Transformation Alimentaire: a consortium mandated by MAPAQ at McGill University in collaboration with CTAQ and MEI. Below is the total budget for the 13 activities of the RITA network.

Budget total : $2,765,828

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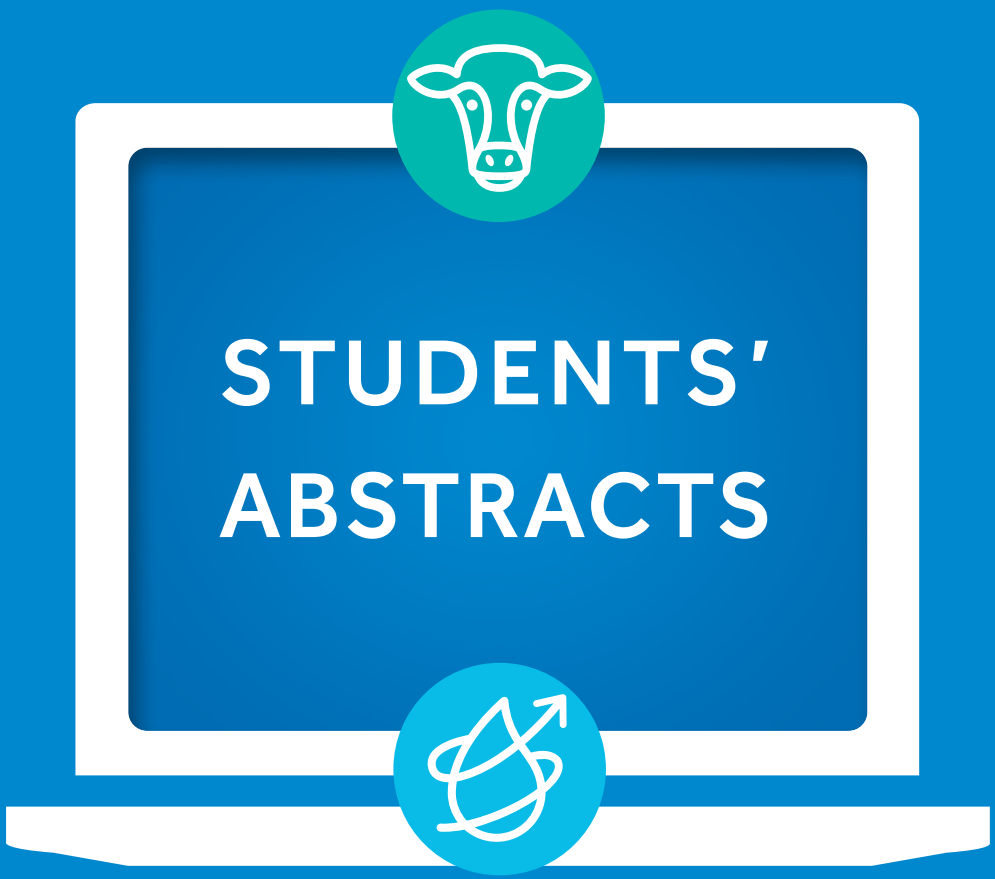

STUDENTS' ABSTRACTS

2021

Novalait

	Student presentation title	Student
PRODUCTION	**Comparison of predictions of milk protein production for Quebec farms by different formulation models**	Simon Binggeli
	Are bacteriocins a natural approach to preventing bovine mastitis?	Samantha Bennet*
	Impacts of milk replacer feeding on the pre-weaning performance of calves	Léonie Laflamme-Michaud
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* CDC Scholarship

Comparison of predictions of milk protein production for Quebec farms by different formulation models

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Protein takes an important part in dairy cow diets, but also lead to pollution when fed in excess. There are a few models commercially available and used to predict protein requirements, supplies and utilization. Comparing the performances obtained for Quebec commercial farms with the predictions offers a unique opportunity to verify how the models perform in the field.

To predict milk protein production, 4 feed evaluation models were selected and coded in R. These 4 models were the National Research Council (NRC) 2001, the Cornell Net Carbohydrate and Protein System (CNCPS) version 6.5.5, NorFor (2011) and INRAtion (2018). Analyses were conducted on 2 diet and production data sets, one smaller but more accurate, one very large (541 and 590 000 production data, respectively). Performance predictions were evaluated based on 2 metrics, concordance correlation coefficient (CCC) and root mean square error (RMSE).

For the first database, the NorFor and CNCPS models proved to be the best models as much using the CCC (NorFor, CNCPS, NRC et INRAtion: respectively 0.82, 0.76, 0.75 and 0.74 – higher values is desirable) or RMSE (NorFor, CNCPS, INRAtion et NRC: respectively 136, 156, 169 and 173 – lower values more desirable). With the largest database, Norfor remains the best model with the 2 indicators while INRAtion and CNCPS share second rank (CCC: NorFor, INRAtion, NRC et CNCPS: 0.72, 0.68, 0.61, 0.59 and RMSE: NorFor, CNCPS, NRC et INRAtion: 194, 224, 235 and 248). From this analysis based on commercial dairy farms, the Scandinavian model seems to be well suited to conditions in Quebec. Changes for a better feed evaluation model on protein utilization will likely lead to a reduction in nitrogen excretion and diet cost, while maintaining productivity.

Are bacteriocins a natural approach to preventing bovine mastitis?

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Using antibiotics to treat and prevent clinical mastitis has certainly helped control this disease in dairy cow production. However, their use has led to the emergence of multi-resistant bacteria that represent a serious threat to both human and animal health. It has therefore become imperative for the dairy sector to develop new alternatives. Bacteriocins are naturally occurring antimicrobial peptides synthesized by a wide variety of bacteria that inhibit the growth of other microorganisms. In this project, the inhibitory activity of various bacteriocins was evaluated in bacteria collected from bovine mastitis cases. Our work showed that bactofencin, nisin, and reuterin can inhibit the growth of most antibiotic-susceptible or multi-resistant *Staphylococcus aureus*, *Streptococcus dysgalatiae* and *Streptococcus uberis*. Next, the efficacy of a teat bath made using bactofencin, nisin and reuterin, alone or in combination, was evaluated in dairy cows. Our results showed that nisin and reuterin, alone and in combination, reduced the bacterial load on the skin of the teats, without causing toxicity or inflammation. To our knowledge, this is the first study evaluating a consortium of bacteriocins to prevent bovine mastitis. The next step in the project will be to evaluate the efficacy of an intramammary infusion combining nisin and reuterin to treat subclinical mastitis in dairy cows. Ultimately, the initial results are promising and suggest that these natural molecules are potential alternatives to antibiotics. Their potential use in dairy production could have many benefits for the dairy industry, such as reducing the costs associated with milk withdrawal during mastitis treatment, as well as limiting the spread of multi-resistant bacteria that can be transmitted to animals and people.

Impacts of milk replacer feeding on the pre-weaning performance of calves

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The National Farm Animal Care Council (NFACC) recommend feeding calf with an amount of milk equivalent to 20 % of their birth weight per day. The aim of this study was to evaluate the impacts of an *ad libitum* feeding (AdLib) compared with the recommended amount (ReCan) on growth and development of 187 calves from two commercial farms in Quebec, Canada. Weaning program was spread over 12 days for ReCan calves and 24 days for AdLib calves, the last 12 days being identical for both. As so, calves were completely weaned at 76 days of age.

Results showed that AdLib had an average daily milk intake of 10,9 L during the 7 days of peak consumption and ReCan consumed on average 8,0 L ($P < 0,001$). Pre-weaning ADG (7-50 d) was higher for AdLib (0,97 vs 0,84 kg/d; $P < 0,001$) than for ReCan. The ADG during weaning (64-76 d) tended to be lower for AdLib (0,92 vs 0,98 kg/d; $P = 0,09$) than for ReCan, and was not different in post-weaning (77-86 d) (1,30 vs 1,26 kg/d; $P = 0,48$). AdLib weight was higher during pre-weaning (90,7 vs 84,7 kg; $P < 0,001$), weaning (116,1 vs 111,7 kg; $P < 0,001$), and post-weaning (130,1 vs 125,5 kg; $P < 0,001$) in comparison to ReCan. For the three periods, withers height and hips height did not differ between treatments. However, hips width was larger for AdLib than ReCan during pre-weaning (22,9 vs 22,3 cm; $P < 0,001$), weaning (25,3 vs 24,8 cm; $P < 0,001$) and post-weaning (26,4 vs 26,1 cm; $P = 0,02$). The growth curve showed that AdLib calves differ by a higher body weight starting on the first weeks of age. Our research demonstrate that producer could reach higher growth and development of their calves by feeding them more milk during pre-weaning.

Impacts of milk feeding on the post-weaning performance of heifers

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The beneficial effects of ad libitum milk diets on the pre-weaning growth performance of heifers are already conclusive. However, the impacts of this type of diet on the post-weaning period have received less attention. The objective of this study was to evaluate whether the effects observed on heifers persist after weaning. On a commercial farm located in Quebec (Canada), 84 Holstein heifers received different milk feeding programs from 7 to 76 days of age and were followed until 11 months of age. Following a randomized distribution, half of the heifers were fed lacto-replacer (28% CP, 16% fat, 15% DM) as recommended by the Code of Practice for the Care and Handling of Dairy Cattle (ReCan: 20% body weight at birth), the other half were fed an ad libitum diet (AdLib). For weaning, the amount of lacto-replacer was gradually reduced over 12 and 24 days for the ReCan and AdLib groups, respectively, where the last 12 days were identical. Weaning was completed at 76 days. The average peak lacto-replacer consumption was 7.8 l/day for ReCan and 9.9 l/day for AdLib ($P < 0.001$). The results showed a higher weight at 11 months for heifers on AdLib compared to those on ReCan (376.4 kg vs 362.8 kg; $P = 0.02$). The hip height of the AdLib group was also higher than that of the ReCan group (136.0 cm vs 134.0 cm; $P = 0.01$). For thoracic circumference, withers height, hip width, and average daily post-weaning gain, no significant effect was noted. Results indicate that pre-weaning gains may persist over time.

Characterization of continuous ruminal pH on 12 commercial farms in Quebec

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Feeding high-producing cows is a delicate balance between providing enough effective fibre and nutrient-rich feeds to maintain good ruminal health, while meeting the animals' elevated requirements. Subclinical ruminal acidosis occurs during episodes of ruminal health disturbance often associated with high starch content in the ration. This disorder has several consequences, such as reduced production and dietary efficiency. As it is difficult to diagnose, little information is available on acidosis on our Quebec farms. Our research project is the first step toward the characterization of continuous ruminal pH using boluses to measure the ruminal pH of 112 cows on 12 Quebec farms.

The project objectives are to characterize the ruminal pH of the cows being studied, to detect subclinical ruminal acidosis events from this pH data, and to correlate it with the fatty acid profile of the cows' milk.

Ruminal pH measured during the study reveals significant variability in the ruminal pH of animals on the same farm. In addition, the average ruminal pH varies greatly from farm to farm. The study of the influence of the various management elements shows a lower average ruminal pH for cows milked by robot and higher for cows receiving monensin ($P < 0.05$). The variation in ruminal pH in the course of the day is very similar for cows from the same farm, suggesting that management has a significant effect on changes in the ruminal pH of cows throughout the day.

At the end of this study, we will have a better understanding of ruminal pH variability that will help us to better situate subclinical ruminal acidosis in our Quebec context. Early detection of subclinical ruminal acidosis could increase dairy company productivity and profitability while improving cows' well-being.

The significant role of *Klebsiella pneumoniae* in clinical mastitis in dairy cattle housed on recycled manure bedding

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The rising costs of traditional bedding have led dairy farmers to explore alternatives. As a result, recycled manure bedding (RMB) is becoming increasingly popular in Quebec. The potential negative health effects of this practice remain largely undocumented but appear to be significant. The objective of this study was to compare the number of clinical mastitis cases in herds using RMB versus those using straw. To our knowledge, this is also the first study to identify the pathogens responsible for clinical mastitis.

A 2018-2019 study conducted on 26 farms using RMB and 60 farms using straw bedding collected milk samples from each area with a clinical mastitis case for one year. The samples were then cultured, and the pathogens were identified.

The dairy farmers submitted 1144 samples during the course of the study. The number of clinical mastitis cases was not statistically different depending on the bedding used (mean [CI 95%]; RMB: 14 [8-24]; straw: 16 [9-30] cases/100 cows per year). However, the number of mastitis cases caused by *Klebsiella pneumoniae* was significantly higher in herds on RMB (incidence ratio [CI 95%]: 7.0 [2.0-24.6]).

Mastitis caused by *Klebsiella pneumoniae* is serious and can put the animal's life at risk. This is a relevant observation for dairy farmers who have adopted the practice or are considering it. The use of RMB may pose health risks to the animals. This project better characterizes these risks and aims to help dairy farmers establish an effective on-farm risk management plan.

Bacteriological screening for endometritis: towards a more judicious use of antibiotics?

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Postpartum endometritis is a disease that affects reproductive performance in dairy cows. Its diagnosis is based on the presence of excessive inflammation in the uterus following calving. In curative medecine, the use of antibiotic treatment in the form of intrauterine infusion in cases of endometritis has been shown to be beneficial in improving reproductive performance. Considering the modern issues concerning the use of antibiotics in animal production, it is justifiable to question the use of a diagnostic method based on the presence of inflammation in order to administer antibiotic treatment. In this sense, the main objective of this research project was to validate the accuracy of the results of bacteriological culture media used on the farm (Tri-plate and Petrifilm) in relation to laboratory bacterial analysis using uterine samples. To do this, a total of 189 cows in the postpartum period (30 to 43 lactation days) were enrolled in order to collect 2 uterine samples with cytobrush. These samples were used to inoculate on-farm culture media, to obtain a bacterial analysis from a reference laboratory as well as to evaluate the inflammation of the uterus at the time of the examination. The results obtained support that the Triplate medium used on the farm allows to obtain a bacterial analysis on the farm comparable to the one obtained in the laboratory. The comparison between bacterial and inflammatory analysis shows a lack of agreement between the two techniques. These results show the potential of the bacteriological approach for the diagnosis of endometritis in cows. Although more projects are needed to confirm our data, it appears that this technique may have the capacity to reduce the amount of antibiotic used and increase the effectiveness of the treatments for endometritis. Reproductive performance would be increased while harmonizing with a more judicious use of antibiotics which would be beneficial for the image of milk production.

Profiling the welfare status of Quebec dairy herds through machine learning and analyzing its impact on performance and profitability

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Animal welfare (AW) is part of sustainability certification programs, but the relationships between welfare and herd performance and economics are unclear, even though the implementation of AW solutions is influenced by their economic impact. Our objective was to describe the welfare status of Quebec dairy herds and analyse its relationship with profitability, longevity, and production metrics. The welfare responses prevalence of lameness, hock, neck and knee lesions, and animals with a body condition score (BCS) ≤ 2, collected between 2016 and 2019, were extracted from the proAction® database from 2696 herds. Lactanet provided profitability (milk value), longevity (length of productive life and percentage of animals on third or greater lactation; 3+ Lact), productivity (energy corrected milk; ECM), and the composite herd status (i.e., welfare and health remote monitoring tool; HIS) metrics. Self-organizing maps and hierarchical clustering were used to profile farms based on welfare responses, HIS, season and barn type. Profitability, longevity, and productivity were compared between the five identified clusters using a random forest model. Cluster 5 (N = 1050) had the best overall welfare status, being composed by herds less likely of having a high prevalence of lameness, hock, neck and knee lesions, and animals with BCS ≤ 2 while being more likely to have a higher HIS value. Farms on cluster 5 were also more likely to have high milk value, 3+ Lact, and ECM. Cluster 2 (N = 234) was more likely to have a high prevalence of BCS ≤ 2 and knee lesions, while cluster 1 (N = 288) had the highest prevalence of neck lesions and cluster 4 (N = 473) had a high prevalence of both hock lesions and lameness. In summary, we segmented herds based on their AW responses and good welfare status were associated with better performance and economic metrics.

Can milk analysis predict the level of well-being of dairy cows?

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Traditionally, assessing the welfare status (WS) of dairy cows required farm visits. Monitoring dairy cows' WS using milk samples already collected for milk recording would be a practical alternative. The aim of this study was to apply a new spectral analysis method combining principal component analysis and mixed modelling to isolate spectral fingerprints representing the effects of stall configuration on milk composition. This new method was used in trials examining the impact of changing stall characteristics – including 4 tie rail (TR) positions and 2 chain lengths – on milk composition. The principal components, extracted from the averages of spectra collected during weeks 8-10, revealed a significant effect of housing factors. Regarding negative WS assessment, spectral analysis revealed higher levels of biomarkers related to body fat mobilization in milk from dairy cows subjected to the most restrictive TR treatment, suggesting a potential hindrance of feed access in these cows. These results were confirmed by a greater prevalence in these cows of injuries at two points on the neck related to access to the feed trough. Regarding positive WS assessment, milk from cows tethered with longer chains had lower levels of biomarkers related to episodes of ruminal acidosis. Behavioural observations showed that these cows spent more time with their heads in the feed trough, assuming that they would have been able to chew more. As a result, they would have produced more saliva to balance the ruminal pH. The new methodology provides a new tool for evaluating dairy cow WS and allows remote detection of cows or herds with welfare problems without farm visits.

Not all potentially more digestible alfalfa is more digestible under our conditions

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Research Context

More digestible alfalfa cultivars, either conventionally selected or genetically modified (GM), are used in Quebec. Improved digestibility has the potential to improve alfalfa's energy content and harvest flexibility.

Research approach

The objective was to compare the yield, quality, and winter survival of more digestible alfalfa cultivars under two cutting intervals in Quebec. Eight cultivars were evaluated: two GM, four conventionally selected, two with low lignin, one with high pectin and one with improved enzymatic stem degradability. There were two controls. They were harvested at the early bud stage or with less than 10% in flower.

Results and applications

All the cultivars survived the winter conditions. In the first year of production, the cultivars had seasonal yields comparable to the controls, except for the cultivar selected for improved stem degradability (-13%). The GM cultivars had an NDFd that was 4.7% units higher and NDF that was 1.0% unit lower than the controls. The conventionally selected cultivars had similar NDFd to the controls. The GM cultivars offered greater harvest flexibility. With one fewer cut per year when harvested at the early flowering stage, GM cultivars had 1.0 t DM/ha higher seasonal yield, similar NDFd, lower crude protein content (-3.1% units), and higher NDF (+5.6% units) and lignin (+0.16 % units) content than control cultivars harvested at the early bud stage.

Potential benefits for dairy farmers

The use of GM alfalfa cultivars may provide greater harvest flexibility and increase ration digestibility, thus potentially improving dietary efficiency in dairy cows.

Protein nutrition: Can we do more with less?

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Research context

The current recommendations for protein requirements for dairy cows are expressed in terms of metabolizable protein, which may result in overfeeding certain amino acids. However, a decrease in metabolizable protein intake is typically associated with a decrease in milk production, probably caused by limiting certain essential amino acids.

Research approach

This project evaluated the impact of varying energy intakes (94, 101, and 107% of requirements) on the performance of dairy cows fed rations with reduced metabolizable protein (86% of requirements on average), but balanced for histidine, lysine, and methionine, three essential amino acids that are often limiting. The control ration was balanced for these essential amino acids and combined 103% of the metabolizable protein requirement and 108% of the energy requirement. Eight early-lactation Holstein cows were fed these four diets alternately for four consecutive 21-day periods.

Results and applications

Compared to the control ration, decreasing the intake of metabolizable protein while maintaining the same energy balance improved the animal's protein utilization efficiency while maintaining energy-corrected milk production, as well as fat and true milk protein production. The three rations that were low in metabolizable protein reduced the cows' nitrogen excretion.

Potential benefits for dairy farmers

It is possible to reduce the metabolizable protein in the ration without negatively affecting dairy cows' productivity at the beginning of lactation, as long as energy and essential amino acid requirements are met. Such changes to the ration help lower the nitrogen emitted into the environment, thereby reducing the environmental impact of milk production.

Natural antimicrobials of bacterial origin: a potential weapon against dairy biofilms

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Lactic acid bacteria produce many antimicrobial compounds, including bacteriocins, organic acids, and biosurfactants. This new generation of natural antimicrobials represents a very promising alternative to chemical disinfectants in the food sector, particularly for controlling biofilms produced by certain heat-resistant lactic acid bacteria (LAB), which have a major impact on the quality of milk and dairy products.

The overall objective of this project is to identify bioprotective cultures that produce antimicrobial compounds that inhibit the formation of dairy biofilms by LABs. One hundred and fifteen strains of lactic acid bacteria belonging to the *Lactobacillus* (n=50), *Lactococcus* (n=22), and *Pediococcus* (n=24) genera were tested against several indicator bacteria. The effectiveness of the metabolites produced by the active strains in preventing or inhibiting the formation of dairy biofilm was evaluated. The minimum inhibitory concentrations for dairy biofilm formation of three bacteriocins (nisin A, bactofencin A, and pediocin PA-1) and two organic acids (lactic and critical) were determined using a microplate assay.

The results showed that 28 culture supernatants were active against *L. ivanovii* HPB28 while eight were active against *C. tyrobutiricum* ATCC 25755. As for anti-biofilm activity, bactofencin and nisin showed significant inhibitory effects on dairy biofilm formation while no effect was observed with pediocin. Citric acid, used at half the MIC, reduced biofilm production by half. However, no reduction was observed with lactic acid. The results show potential for the use of bacteriocins in controlling the formation of dairy biofilms. They open the door to industrial applications in the dairy industry, enabling better control of this phenomenon and of economic losses.

Persistence of lactic acid bacteria strains in a dairy farm's milk pipeline

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For several years, it has been demonstrated that on-farm management practices modify the microbiota of raw milk, which has an impact on dairy product quality. Until now, the milk contamination route and the factors favouring certain bacteria have not been well known. The hypothesis of this project is that the modification of the milk microbiota is partly due to the development of biofilm on the surface of the milking system equipment. It appears that the lactic acid bacteria (LAB) abundant in raw milk are all indicated for contributing to the development of these biofilms, in particular through the production of exopolysaccharides composing the biofilm. The objective of the study was to detect and enumerate LABs in the milking system of a dairy farm during two visits at a one-year interval. Various surfaces of the milking system (bulk milk tank, milk filter location, milk pipeline and milking machine) were sampled. The LABs were counted and isolated on a selective agar. The presence of LABs in various locations differed between the two samplings. *Lacticaseibacillus casei*/paracasei and *Pediococcus acidilactici* strains were present in the milk pipeline in both years. Surprisingly, these strains did not demonstrate the ability to produce biofilms in milk, nor the ability to produce exopolysacharrids. It is possible that these strains adhere to the surface by other mechanisms or that biofilm is produced by other microorganisms. This work shows that the development of dairy biofilm in the milking system such as the milk pipeline is not negligible considering the persistence of LAB strains over a period of one year. Better identification of persistent bacteria in the milking system will help optimize the cleaning methods used on the farm to improve milk quality.

Production of dairy biofilms on stainless steel surfaces in a bioreactor and development of an efficient sampling method

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The dairy industry could face disturbing situations due to the presence of biofilms in its dairy processing plants (DPP) in which their existence substantially could reduce the safety and quality of dairy products, leading to significant economic losses. Cleaning in place is the current method used in DPP to control biofilms but unfortunately, it does not always guarantee their total elimination.

In this context, the acquisition of new knowledge related to the dairy biofilms constitution on the chemical, structural and microbiological levels would allow a more efficient control over them.

The first part of this research project aims to: (1) Optimize the conditions for the development of dairy biofilms on stainless steel surfaces in a dynamic bioreactor system and (2) Develop a sampling technique adapted for the DDP.

Our results demonstrated the formation of strong biofilms under dynamic conditions by the dairy-isolated *Pseudomonas fluorescens* PFl1A strain in culture medium (TSB) and milk with bacterial counts of 8.42 and 7.74 log CFU/cm^2, respectively. Furthermore, the sampling method combining sonication and synthetic sponge allows an efficient removal of biofilms and a recovery similar to that obtained with ultrasonication (ASTM E2871-19). By its flexibility and its convenience of use in the DPP, it would allow an adequate sampling of biofilms.

In conclusion, our research will enable us to develop methods and tools reaching a better study and characterization of dairy biofilms and eventually propose control strategies through the development of new adapted antibiofilm formulations. Hence, Quebec's DPP will ensure safe and high-quality dairy products.

Monitoring lactococcal phages over 20 years in a cheese factory

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Since the 1930's, bacteriophages of lactic acid bacteria have been extensively studied. By infecting and killing starter strains, virulent phages can slow down the milk fermentation process leading to low quality fermented dairy products. Phage populations are usually monitored in large factories to adapt anti-phage measures such as starter rotation in order to avoid production delays and to ensure product uniformity.

For two decades, our research group has been studying the lactococcal virulent phage population within a cheddar cheese plant in the Province of Québec. This university-industry collaboration led to the collection of 889 isolates and 241 novel phages infecting various industrial strains of *Lactococcus cremoris*. First, reduced starter activity was detected in whey samples at the dairy processing plant. Then, the positive samples were sent to the Université Laval for further analysis. Lactococcal virulent phages were isolated and characterized to distinguish them and identify their viral genus. A host range was subsequently realized on *Lactococcus* strains used in the cheese manufacturing facility.

Of the 889isolates, 97% (861) were found to belong to the *Skunavirus* genus making it the most predominant viral genus in this cheese factory. Specific genomic regions of these phages were sequenced and then analyzed phylogenetically. These lactococcal phages clustered according to the hosts they infect rather than their year of isolation. Interestingly, some phages were isolated over several years while others were seldomly isolated. Also, these virulent phages were found to be specialists as they are mostly host specific, rarely infecting more than one bacterial host. These results support the practice of strain rotation currently used in the dairy industry, thereby limiting cheese losses. It also indicated that novel phage resistance mechanisms should target this viral genus.

Proteins of lactic bacteriophages: a great mystery

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Streptococcus thermophilus is one of the most widely used bacteria by the dairy industry. This lactic acid bacterium is primarily used in starter cultures for the production of yogurt and speciality cheeses. Like all bacteria, strains of *S. thermophilus* can be inactivated by bacteriophages or phages. These bacterial viruses represent a significant risk, particularly to the cheese industry, as they can negatively impact cheese quality. The study of phage biology is an essential step in adapting anti-viral strategies and in limiting the emergence of novel phages in industrial settings.

A third to half of the early expressed genes in the genome of phages infecting lactic acid bacteria encode proteins of unknown functions. In general, viral genes that are readily expressed at the beginning of the infection encode proteins that interact directly with bacterial molecules. The main objective of this project was to investigate some of these phage proteins. Because the binding partner of a given protein usually reflect the latter's function, we are developing methods based on affinity purification to study protein-protein interactions using the model phage 2972 and its host *S. thermophilus* DGCC7710.

Phage mutants were generated using the CRISPR-Cas9 genome editing tool. These mutants encode a tagged (SH-tag) viral protein of interest. We believe that affinity purification, using the intracellular content of phage-infected bacterial cells, will allow to recover the tagged proteins and its molecular partners, hence allowing us to study the protein-protein interactions involving the protein of interest. However, the lysis and the proteome extraction of *S. thermophilus* cells were surprisingly challenging during the development of the method. Indeed, this Gram-positive bacterium was resistant to many standard lysis techniques. Among the tested strategies, purified phage endolysins were the most promising in lysing the DGCC7710 strain.

Impact of the native microflora of Quebec cheeses on texture

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Cheese texture is one of the easiest quality criteria for consumers to recognize. This texture can be modified both by technological levers and by the activity of the microorganisms present. During the study of the natural microbiota of Quebec cheese, several bacteria, yeasts, and moulds were isolated and identified. These microorganisms are of interest to cheesemakers for their possible role in the development of typical sensory properties (taste, odour, texture). The primary objective of this project is to assess the still little-known impact of this secondary microbiota on cheese texture.

Bacterial strains of *Lactobacillus plantarum*, *L. curvatus*, and *Staphylococcus equorum*, as well as yeasts of the species *Cyberlindnera jadinii*, *Pichia fermentans*, *Kazachstania servazzii*, *Pichia kudriavzevii*, and *Rhodotorula mucilaginosa* were analyzed for their proteolytic and lipolytic abilities on calcium caseinate and tributyrin environments, respectively, and incubated at 15°C and 25°C for 21 days. The strains' activities were evaluated by measuring the halo generated when the compounds were used around the colonies. The results show that *Lactobacillus* are highly proteolytic at 15°C. As for *S. equorum*, it seems to have moderate proteolytic and lipolytic activities at any temperature. Among the yeasts, only *K. servazzii* showed proteolysis at 25°C, but most of the yeasts had lipolytic activity. These technological characteristics are indicators of a potential change in cheese texture.

For the rest of the project, the highly proteolytic strains will be individually added to cheeses whose physico-chemical changes - primarily in texture - during ripening will be evaluated. This new knowledge will provide a better understanding of the impact of secondary microbiota on cheese texture, and thus ensure better quality control of Quebec cheeses.

Lactoferrin, lactoperoxidase, and lysozyme content in cheese-making milk

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Variations in cheese quality can be explained by several factors, such as diet, breed, season, manufacturing practices, starter cultures and the bacterial microbiota of milk. Antimicrobial proteins are among the factors that influence the microbiota. This project aims to determine the concentration and activity of lactoferrin (LF), lactoperoxidase (LP) and lysozyme (LZ) in commercial cheeses. An enzyme-linked immunosorbent assay (ELISA) was used for each protein. In addition, LP was measured by spectrometry, LZ by fluorescence, and LF by HPLC.

Camembert cheese had systematically less LP, LF and LZ than the other 3 cheeses. The two LP analytical methods allowed the determination of LP activity itself, which should have generated similar results. However, there was no strong correlation between the results of the two methods ($R = 0.46$; $p = 0.13$). LP activity results are therefore a function of the methodology used. As for LZ, the spectrophotometric method gave the activity while the ELISA method revealed total protein-LZ content, regardless of the activity (ELISA). There was no correlation between the total amount of LZ and the residual amount that had activity. This reveals that activity losses differed between cheeses. The two LF analyses gave the protein content in the cheeses. As with LP, there was a certain correlation ($R = 0.40$) but not a strong one ($p = 0.2$).

These results show that antimicrobial milk proteins are found in cheese, but at varying levels. Studies on their role in the evolution of the microbiota during maturation are underway.

Impact of adding sodium caseinate on the cheese-making properties of reverse osmosis milk concentrates

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The use of reverse osmosis (RO) milk concentrates would reduce the costs associated with transporting milk and increase yield by increasing the amount of serum protein and lactose in the aqueous phase of cheese. However, due to excessive mineralization of the casein micelles, RO concentrates are rarely used in cheese production. Adding sodium caseinate (NaCas) to RO concentrates would chelate ionic calcium and restore the mineral balance in cheese milk. This study aims to characterize the impact of the concentration of added NaCas on the cheese properties of skimmed milk concentrates.

Model RO concentrates were prepared from skimmed milk powder, rehydrated to two levels of total casein (TC) (4.5 and 6%). A portion of the casein was substituted with NaCas in four proportions (0; 6; 12; 18%). The soluble-colloidal balance of the model concentrates was characterized as well as their cheese-making properties. The soluble phase was recovered by ultracentrifugation and the mineral composition was determined by ICP-EOS. Model cheeses have also been produced to assess protein and fat retention coefficients, moisture, and the rheological and melting properties of cheeses.

The increase in the proportion of NaCAS significantly demineralizes ($p < 0.05$) the casein micelles in RO concentrates. The cheese moisture decreases and the true protein retention increases significantly ($p < 0.05$) with the increase in the proportion of NaCas. The addition of NaCas is a valuable approach to 1) demineralize the casein micelles; 2) improve cheese mass balances; and 3) improve the cheese-making properties of RO concentrates by allowing better contraction of the curd. By facilitating the use of RO concentrates in cheese making, the addition of NaCas can provide economic and environmental benefits for processors.

When buttermilk is under high pressure

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Buttermilk (BM), a co-product of the butter industry, is distinguished from skim milk (SM) by its richness in phospholipids (PL) from the fat globule membrane (FGM). Despite their numerous biological activities, PLs alter the techno-functional properties of BM. The use of baromembrane processes for the fractionation of BM PLs to obtain a product with high nutritional value is of great interest. However, the similar size of FGM fragments and casein micelles (CM) complicates their separation. This project aims to study the effect of ultra-high homogenization pressure (UHPH) and pH on these components to create a size gradient for their subsequent separation. BM (pH 6.7) was adjusted to pH 8.5 and 10.5, homogenized to 100, 200 and 300 MPa and compared to SM. Changes in particle size were monitored by laser diffraction, and structural changes and protein distribution by turbidity and electrophoresis gels. At 0 MPa, an increase in mean particle diameter ($D_{4,3}$) was observed from 366 nm (pH 6.7) to 747 nm (pH 10.5). Therefore, UHPH results in a reduction of $D_{4,3}$ to 328 nm at pH 10.5. Turbidity also decreases drastically with alkalinization and even more with UHPH treatments. Finally, electrophoresis gels demonstrate that UHPH promotes separation of CMs previously destabilized by alkalinization. Similar results were observed with SM, suggesting that the impact of pH and UHPH is predominantly on CMs. This study demonstrates the potential of combining UHPH and alkalinization to modulate the size of CMs and facilitate their separation from FGMs using baromembrane processes. Fractionating BM will generate two high value-added components: a bioactive component rich in PL from the FGM and a delipidated techno-functional component that is rich in protein and can be used in dairy products.

Impact of ultra-high-pressure homogenization of buttermilk for the production of yogurt

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Buttermilk (BM) is the by-product of a cream churning process for butter-making. It is used in various food formulations (e.g., bakery, snacks) where it competes with powdered milk or whey. Although its composition is similar to skimmed milk (SM), certain BM characteristics limit its use technologically, notably the higher phospholipid (PL) composition. Studies have shown that the functional properties of BM are therefore often inferior to SM except for its emulsifying properties. However, pre-treatment of BM could be used to improve its techno-functionality. Therefore, the objective of this study was to investigate the impact of ultra-high-pressure homogenization (UHPH) on techno-functional properties of BM in yogurt manufacturing. Buttermilk and SM (control) were treated by conventional (15 MPa), high pressure (150 MPa) and UHP homogenization (300 MPa) before yogurt production. Physico-chemical analyses and textural characterization were performed on both yogurts. The result showed that for set yogurts, treatment of BM by UHPH resulted in the formation of a weaker gel, whereas higher firmness was observed for SM yogurts. On the contrary, SM set yogurts had a gel microstructure characterized by large protein clusters surrounding serum pools, compared to the homogeneous gel observed for BM. While the use of BM and UHPH lowered the apparent viscosity in stirred yogurts, they were significantly less prone to syneresis than SM. PAGE analysis showed that UHPH induced higher protein denaturation in BM than SM which could explain this decrease in syneresis. Overall, the use of UHPH on BM has great potential for low viscosity yogurt applications such as ready-to-drink yogurts and other fermented beverages. Valorization of BM into dairy products will allow the dairy industry to increase its profit by simultaneously benefiting from reducing food waste and the production of health promoting products rich in BM.

A social affinity test for lactic acid bacteria

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Dairy products such as grated cheese, yogurt or pasteurized milk are environments that are conducive to the development of microorganisms that limit their shelf life. Breaks in the cold chain as well as contamination by our hands or by the environment accentuate spoilage and modify the organoleptic properties of the product. Spoilage leads to economic losses for both the manufacturer and the consumer. In almost all environments, microorganisms do not live isolated, they cohabit with other microorganisms. This coexistence gives rise to interactions that can play a determining role in the alteration of food. However, these microbial interactions and their roles in food spoilage are not well known.

The aim of our study is to characterize on a large scale the social relationships between microorganisms of the dairy processing environment. To this end, a method was developed using a robot to measure microbial interactions between lactic acid bacteria and isolates from dairy products.

We were able to identify different social personalities in lactic acid bacteria. For instance, some strains favor the growth of other lactic acid bacteria at the expense of dairy isolates, while other strains adopt the opposite behavior. Measuring the effect of microbial interactions in various contexts will allow us to develop a predictive tool that will consider microbial interaction effects. Ultimately, the methods developed will allow us to identify combinations of strains with desirable behavior for various applications and to avoid combinations that may lead to quality problems. For example, consortia of bio-protective cultures capable of naturally extending the shelf life of dairy products could be developed. In the long term, the results of this study could contribute to the reduction of food waste.

Using arctic bacteria to help preserve cheeses

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Fungal cheese spoilage causes a significant amount of food waste leading to large economic losses in the cheese industry. One of the methods the cheese industry uses to combat fungal spoilage is the use of natamycin, which is a type of polyene that inhibits fungal growth. Antimicrobial resistance towards polyenes is rising and consumer demand for natural, "Clean Label" products is increasing, leaving very few viable alternatives. This project focuses on identifying novel antifungals from arctic bacteria that can be used in the cheese industry at refrigerated temperatures. The project was broken down into 3 objectives: 1st *in silico* search for antimicrobials; 2nd *in vitro* screening of arctic bacteria; and 3rd extraction and identification of antifungal compounds. We searched in silico for antifungal proteins and secondary metabolites using a collection of 146 whole genome sequences of arctic bacteria. We found a variety of gene clusters producing secondary metabolites, chitinases and glucanases. The collection was then screened *in vitro* for antifungal activity using co-culture screening techniques between the arctic bacteria and fungal strains common to the dairy industry. The most promising isolates were assessed for novelty based on sequence data and resulted in no known antifungal genes. To identify the active component(s), organic extractions using ethyl acetate were conducted to produce crude extracts, which after optimization allowed for the inhibition of all fungal strains tested in this study. The extracts were then analyzed using LC-MS to obtain a list of suspected bioactive agents; however, several unknown compounds were found. The next steps would be to conduct preparative HPLC to purify and determine the active component. In the long term, this project has the potential for several valuable outcomes including reducing economic losses due to fungal spoilage while at the same time meeting consumer demands for more "Clean Label" products.

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