FORUM TECHNO 2018

Novalait

Research Catalyst

A Word from the President

On behalf of the Board of Novalait, it is with great enthusiasm that I welcome you to the Forum Techno.

Our goal is to immerse you in the research experience. The 2018 Forum Techno offers a selection of the best solutions and discoveries of our scientific teams. Researchers and students share a passion for dairy research through conferences, workshops and poster presentations.

Although research has its share of uncertainties, the relevance of Novalait's projects is not a coincidence. Initially, Novalait's research priorities are established by dairy farmers and processors. Novalait appeals to the scientific community to develop innovative solutions. Proposals are selected based on benefits for the dairy industry and scientific quality. On several occasions during the project, user committees question researchers about their findings and discuss the applications of the results. Novalait explores opportunities to protect and exploit the developed intellectual property. Each project is subject to a specific strategy to communicate research results to farms and dairy plants.

Dairy farmers and processors have proven that they were visionary in creating an R&D investment company to fuel innovation in their businesses. Starting from one cent / hl, excellent governance and effective management, Novalait has developed a research portfolio whose value now exceeds $ 45 million. Thanks to the partnerships developed, Novalait has enabled the dairy industry to obtain its share of public funds for research. These investments have also attracted and maintained in Quebec brains to meet the scientific challenges of the dairy industry. A whole generation of professionals, at the service of innovation on the farm and at the plant, have been trained in Novalait's applied researches.

The Forum is a unique opportunity to leverage your research investments. Take the opportunity to ask researchers about the applications of their discoveries, to share your research interests and who knows, to recruit new skills to innovate!

To all of you, I wish you a fruitful Forum!

Charles Langlois
President of Novalait

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Program

Wednesday, May 16, 2018

7:15	**Breakfast Conference CRIBIQ - MITACS – CINTECH (French only)**	
8:45	**Welcoming of participants**	
9:00	**Welcoming remark**	
	Discoveries	
9:10	**Biovalorization of whey and permeat by fermentation** Michèle Heitz, U. de Sherbrooke and Stéphane Godbout, IRDA	**Controlling the energy balance through milking rather than diet** Simon Dufour, U. de Montréal
9:35	**Microbial quality of Greek yogurt and valorization of co-products** Gisèle LaPointe, U. Guelph and Claude Champagne, AAFC	**Improving fodder grass in the context of climate change** Gaétan Tremblay, AAFC
10:00	**Quality of stirred yogurt; stability and rheological properties** Sylvie Turgeon, U. Laval and Daniel St-Gelais, AAFC	**New Chair on Sustainable Farm Buildings** Sébastien Fournel, U. Laval
10:25	**Health break – Poster session**	
11:10	**Flash results: 3 minutes thesis presentations by grad students**	
11:40	**Premiere: Preview of the new edition of the book *Sciences et Technologies du lait***	
11:50	**Lunch – Poster session**	
13:45	**Experience the research: demonstration workshops**	
3 X 30 minutes	**Workshop 1** **Eco-efficiency in plants: a new simulation tool for you!** Yves Pouliot and Scott Benoit, U. Laval	**Workshop 2** **Using bioprotective lactic acid bacteria in milk production and processing** Ismail Fliss and collaborators, U. Laval
		Workshop 3 **Practices to improve the comfort and welfare of cows** Elsa Vasseur and collaborators, U. McGill
15:30	**How silage and raw milk affect dairy product quality** - Denis Roy, U. Laval	
	Closing remark	
15:55	**Excellence awards, and closing remark** Charles Langlois, president of Novalait	
16:10	**Wine and cheeses**	

FACT SHEETS

2018

Novalait

Biovalorization of whey and permeat by fermentation

Duration: 2013 – 2017

Highlights

- This project focuses on the transformation of lactose contained in whey and permeate into diols (2, 3-butanediol (BD) or acetoin (A)) through a fermentation process named Biobac. This process creates a genetically superior bacterium to support the metabolic pathway necessary for synthesizing BD and A.
- The project consists of 3 parts:
  - The genes facilitating the production of BD or A were chemically synthesized and added to the chromosome of the *Escherichia coli* bacterium. Mutations inactivating the key genes of five competing fermentation pathways were generated, supporting the metabolic pathway of these two products.
  - The results of laboratory-scale whey or whey permeate fermentation provide yields of approximately 0.4g BD and 0.10g A per gram of lactose.
  - This eco-efficiency study allowed us to identify the economic benefits of Biobac compared with other valorization processes. The price of diols is higher than that of skimmed whey, whey powder and even methane. However, environmentally speaking, there is more work to be done regarding the separation of diols. Based on the results of the life cycle analysis, this separation step is responsible for 60% of the greenhouse gases emitted and 70% of the energy demand.

Objectives

GENERAL OBJECTIVE: Genetically modify a bacterium using a single-step fermentation process, in order to produce two added-value products and to study the product's environmental impacts and energy efficiency.

- Using metabolic engineering, develop bacterial strains of Escherichia coli able to produce molecules of commercial interest such as acetoin and/or 2, 3-butanediol. Validate the application to valorize cheesemaking co-products such as whey or its permeate, containing a high concentration of whey that can be fermented.
- Optimize fermentation of whey and its permeate on a laboratory scale in different operating conditions in the presence of a modified bacteria.

Results and potential benefits

The research has allowed us to identify three coding genes for enzymes able to successively transform pyruvate produced through glycolysis into acetolactate, then into acetoin and, lastly, into 2, 3-butanediol. These genes were synthesized and then integrated into the chromosome of *Escherichia coli*, which made production of the molecules possible. A series of deletions in five fermentation pathways was then developed, which could potentially compete with the production of acetoin and 2, 3-butanediol. The addition of the 2, 3-butanediol metabolic pathway in combination with the deletion of certain fermentation pathways endogenous to E. coli produces the highest yields during the fermentation of glucose. However, the diol yields remain modest (15-20 g/L).

In regards to the fermentation of whey (25 g/L) (on the laboratory scale in the presence of M9 and urea, Mg and Ca, at a temperature of 37 °C, an initial pH of approximately 7, and under atmospheric pressure), BD yield is 0.35g BD per gram of lactose after 72 hours of fermentation, while it is 0.25g BD per gram of lactose after 24 hours. Under identical operating conditions, BD yield obtained from whey and its permeate is 0.40g per gram of lactose.

The environmental and eco-efficiency studies have demonstrated that: a) the valorization of dairy co-products through the Biobac bioprocess provides an alternative sustainable source of energy supply compared to oil with a 51% reduction in greenhouse gases and a 95% reduction in the energy demand; b) the environmental impact of valorizing whey permeate is greater than that of valorizing whey due to the concentration of lactose in the co-products; c) the weaknesses of the Biobac bioprocess are linked to water consumption and the energy demand of the separation process; d) the production of diols (BD, A) has great economic potential thanks to their market prices; and e) the Biobac process helps improve quality of life and better protect natural resources (water) by reducing BOD and COD in industrial wastewater produced during dairy processing.

Professionals trained

Angela Maria Trivino, master's student in agri-food engineering (Université Laval).
Expertise: Environmental impact assessment, life cycle assessment, whey valorization processes, challenges and opportunities in the dairy sector.

Jean-François Rousseau, master's student (Biology, Université de Sherbrooke).
Expertise: metabolic engineering, molecular biology. Professional interest: entrepreneurship.

David Fernandez, PhD student (Chemical engineering, Université de Sherbrooke).
Expertise: fermentation, bioreactors, biotechnological engineering, water treatment.

For further information

- A literature review on the production of butanediol through fermentation was published: Fernandez et al., *Biovalorization of saccharides derived from industrial wastes such as whey: a review*, Journal Reviews in Environmental Science and Bio/Technology, Volume 16, Issue 1, pp 147–174, 2017.
- International and national conferences: a) Fernandez et al. Potential fermentation of a dairy waste into acetoin by *Escherichia coli*, The IAFOR International Conference on Sustainability, Energy and the Environment, Honolulu, Hawaii, January 5-7, 2017; b) A. Trivino et al. (2017) Analyse environnementale des options pour la valorisation du lactosérum, poster session; STELA 2017 symposium, "Accroître notre compétitivité face à la mondialisation." Quebec City, Canada. May 29-31, 2017; c) A. Trivino et al. Environmental study of valorization of cheese dairy residues, Canadian Society for Bioengineering (CSBE/SCGAB) 2016 Annual Conference, Halifax World Trade and Convention Centre, July 3-6, 2016.
- An article describing the development of *Escherichia coli* strains capable of producing acetoin and butanediol is being prepared (Rousseau et al.).
- An article describing the selection of culture media in the presence of one of the modified strains has been submitted (Fernandez et al., 2017).
- Presentations at Novalait's 2016 and 2018 Forums.

Financial contributions

Partnership for innovation in dairy production and dairy processing (EPI2011-2017):

- Fonds de recherche du Québec – Nature et technologies
- Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
- Novalait

Total budget: $220,000

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Microbial quality of Greek yogurt and valorization of co-products

Duration: 2014 – 2018

Highlights

- Greek yogurt has won the favour of consumers and now makes up the largest segment of the yogurt market.
- The dairy industry is in need of comparative data on the stability and safety of these types of high protein content (HPC) yogurts, produced by centrifugation or ultrafiltration, as well as valorization strategies for co-products.
- Cold HPC products contain more probiotics, but their subsequent stability is affected by the strain and the HPC process.
- The survival rate of the sensitive probiotic strain (*Bifidobacterium longum* ssp. *longum* R0175) is not higher in HPC yogurt in comparison to traditional yogurt.
- The centrifugation process concentrates bacteria and curd, furthering survival of the resistant probiotic strain (*Lactobacillus helveticus* R0052).
- Mortality of the contaminant *E. coli* was higher in HPC yogurt produced through ultrafiltration while growth of the yeast *Kluyveromyces marxianus* was similar in HPC and traditional yogurts.
- The production of expolysaccharides by lactic acid bacteria increased in the co-cultures of *Lactobacillus rhamnosus* and the yeast *Saccharomyces cerevisiae* in comparison to the monoculture, making it possible to obtain a bioingredient that is rich in polysaccharides from the lactic acid bacteria ultrafiltrate.
- The solid-state fermentation of whey permeate with wheat bran made it possible to increase the presence and bioaccessibility of the phenolic componenents and antioxydant activity of bioingredients.

Objectives

GENERAL OBJECTIVE: contribute to the advancement of knowledge of high protein content (HPC) yogurt, as well as enrichment processes and microbial communities that impact the quality and functionality of dairy products. The project aims to increase the energy efficiency of processes with the development of high value-added ingredients using co-products in the production of HPC yogurt.

Results and potential benefits

New knowledge:

Effect of the processes on the development of starter cultures, probiotics and contaminants, as well as on sensory characteristics during the storage of HPC yogurts:

- The survival rate of the sensitive probiotic strain (*Bifidobacterium longum* ssp. *longum* R0175) is not improved in HPC yogurt in comparison to traditional yogurt.
- The centrifugation process concentrates bacteria and curd, furthering survival of the resistant probiotic strain (*Lactobacillus helveticus* R0052).
- The mortality rate of the contaminant *E. coli* was higher in HPC yogurt produced through ultrafiltration while growth of the yeast *Kluyveromyces marxianus* was similar in HPC and traditional yogurts.

Development of high value-added ingredients using co-products:

- The production of exopolysaccharides in whey permeate is higher when using yeast and exopolysaccharide-producing lactic acid bacteria co-cultures.
- The combined fermentation of dairy and grain products produces a new ingredient that is richer in antioxidant activity.

New process

Eco-friendly design of a process for biological recycling of the whey ultrafiltrate to produce new bioingredients with prebiotic effects.

Results and potential benefits, continue...

Potential Benefits

- Increase the value of by-products and dairy products fermented with probiotics.
- Increase the energy efficiency of milk processing through biological recycling.
- Better choice of processes depending on the desired product (sensorial or microbiological attributes).
- Decreased energy use and reduced loss of co-products.
- Improved functionality of dairy products for consumer health.

Professionals trained

Andréanne Moineau-Jean, a Master of Science student, has acquired expertise in yogurt production and concentration processes; growth, survival and selective counting in milk, yogurt with lactic acid bacteria, probiotics, and contaminant microorganisms; physico-chemical analyses of milk and yogurt; and the sensory assessment of yogurt. Her professional interests include food processing through the use of microorganisms, the development of foods that are beneficial to health, improving food safety and preservation; and the valorization of co-products resulting from food processing. Her work on the contamination of Greek yogurt during storage earned her the title of star student-researcher of the month for February 2018, awarded by the Fonds de recherche du Québec - Nature et technologie (FRQNT).

Annalisse Bertsch, a PhD student, has acquired expertise in co-culture fermentation processes, bioingredient production, gene expression through RT-qPCR, the viability of strains through PMA-qPRC, and the valorization of food industry co-products. Her professional interests include research and development of products that are beneficial to health (prebiotics and probiotics), and the design and development of energy efficient technological processes.

The training of the two students was considered relevant by the company Biena specializing in the production of lactic and probiotic cultures because Andréanne has been working in the laboratory since graduation, while Annalisse is part of Industrial production team since July 2017.

For further information

The results were presented by Andréanne Moineau-Jean through posters at the Novalait Forum (June 2, 2016, Drummondville) and at IUFoST, 18th World Congress of Food Science and Technology (August 22–25, 2016, Dublin, Ireland). In 2018, the results will be presented by Annalisse Bertsch through an oral presentation and a poster at the Novalait Forum Techno.

One scientific article has been published to date: Moineau-Jean, A., Guévremont, E., Champagne, C. P., Roy, D., Raymond, Y., & LaPointe, G. (2017). Fate of *Escherichia coli* and *Kluyveromyces marxianus* contaminants during storage of Greek-style yogurt produced by centrifugation or ultrafiltration. International Dairy Journal, 72, 36-43.

Financial contributions

Partnership for innovation in dairy production and dairy processing (EPI2011-2017):

- Fonds de recherche du Québec – Nature et technologies
- Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
- Novalait

Contribution to the training course of Myriam Laberge by the Canada Research Chair in Lactic Cultures Biotechnology for Dairy and Probiotic Industries

Total budget: $190,000

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Quality of stirred yogurt; stability and rheological properties

Duration: 2013 – 2017

Highlights

- The province of Quebec produces more yogurt (80%) than anywhere else in Canada.
- A number of factors determine yogurt properties, including the type of bacteria, milk composition (total solids, fats, serum protein/casein ratio) and the production process conditions.
- To date, most research has been completed on firm yogurt, whereas in Canada, stirred yogurt is mainly consumed. Stirred yogurt is obtained by breaking the gel after fermentation in tanks.
- During the conditioning process (stirring, pipe flowing and pumpage), the product is subject to shearing constraints which result in the destructuring of the protein matrix affecting the rheological properties. Once it has been packaged, the stirred gel is stored at a cool temperature and the gel particles can once again interact to partially reestablish the gel properties. At the time of consumption, the yogurt must have textural properties that are acceptable to consumers.
- Results obtained: several critical points during yogurt production were identified regarding resistance to shearing and the capacity to reform a quality gel during storage.
- Anticipated benefits: Equip the industry with decision-making tools to optimize the production process and increase the quality of stirred yogurt.

Objectives

- Our hypothesis is that the composition and conditioning stages of stirred yogurt affect the final yogurt characteristics.
- Our objectives are as follows:
  - To determine the effect of curd shearing during the production process on the rheological properties and quality of yogurt.
  - To determine the effect of the dairy mix composition on the sensitivity of curd to shearing and on its rheological properties.
  - To determine the impact of production parameters and cooling speed on the rheological properties of stirred yogurt.

Results and potential benefits

Importance of composition: resistance to shearing and capacity to reform a quality gel during storage

- Results: Composition determines the properties of stirred yogurt. A high fat content reduces syneresis and increases firmness and viscosity when stored at 4°C. The source of serum proteins (whey protein isolate vs. milk protein concentrate) has a slight effect on syneresis and varies depending on the smoothing temperature. Reduction in casein ratio: serum proteins increase yogurt firmness and viscosity and reduce the serum mobility.

Identify critical points during yogurt production

- Results: Increased shearing during conditioning (test) reduces firmness and viscosity except in the presence of a high fat content. For fat-free yogurt, it would be preferable to reduce shearing intensity. For high-fat yogurt, increased shearing reduces syneresis. The test has demonstrated that only smoothing and cooling had an impact on syneresis, firmness and viscosity. During storage, stirring speed also had an impact. The sequence of the condition stages is important; different properties are obtained depending on whether smoothing is completed before or after cooling.

Improve quality control for stirred yogurt; supporting the development of optimal industrial practices

- Outcomes: Better control of production parameters and increased quality of stirred yogurt (increased consumer satisfaction, decreased downgrading of products, increased profitability). Requires a validation project conducted in-plant. Improved efficiency development process for new products as it will be based on a better understanding of the systems.

Professionals trained

Two master's students graduated during this project.

- **Noémie Lussier** (MSc#2: obj. 2.2) completed a thesis entitled [translation] "Role of fat content and fermentation speed on the development of the rheological properties of yogurt during stirring and storage." Expertise: Physical chemistry, microbiology.
- **Marc-Olivier Leroux** (MSc#3: obj. 3) completed a thesis entitled [translation] "Impact of cooling speed on the stability and rheological properties of stirred yogurt." Expertise: Physical chemistry, process.

One master's student was fast-tracked to a PhD program (**Valérie Guénard-Lampron** MSc#1: obj. 1.2). Her thesis is entitled [translation] "Characterization of the rheological, physical and microstructural properties of dairy microgels: Impact of smoothing temperature and protein ratio." Expertise: Physical chemistry, process

Another PhD student is also being trained in this project.

- **Audrey Gilbert** (funded in part by the project) (PhD#1: obj. 2.1) is completing a thesis entitled [translation] "Impact of process dynamics and the composition of fermented dairy products on their functional properties." Expertise: Physical chemistry, process.

All four students plan to work in the dairy industry after completing their studies. They chose this project for the high degree of applicability of the results and its significance to the dairy sector.

For further information

Posters

- Gilbert A., Rioux L-E., St-Gelais D., and S. L. Turgeon 2018. Whey protein content and smoothing temperature are tools to modulate stirred yogurt structure and rheological properties. 3rd Food Structure and Functionality Forum Symposium & the 3rd IDF Symposium on Microstructure of Dairy Products. Poster. Montreal, Quebec City, Canada, June 3–6, 2018.
- Guénard Lampron, V., S. Villeneuve, D. St-Gelais, and S.L. Turgeon 2017. Stirred yogurt smoothing at different temperatures with a pilot-scale unit. Poster. IDF World Dairy Summit, Belfast Waterfront, Northern Ireland, October 29 to November 3, 2017.
- Leroux, M.-O., S. Villeneuve, M. Reza Zareifard, S. Grabowski, D. St-Gelais and S.L. Turgeon. 2017. Effet de la vitesse de refroidissement et de la teneur en matières grasses sur les propriétés rhéologiques et physico-chimiques de yogourts brassés. STELA 2017 symposium, Accroître notre compétitivité face à la mondialisation. Poster #15. Quebec City, Canada, May 29–30, 2017.
- Lussier, N., D. St-Gelais, S. Grabowski, S. Villeneuve and S.L. Turgeon. 2016. Study of the effects of shear treatment, fat content and fermentation time on rheological properties of stirred yogurt. IDF World Dairy Summit. Poster #WDS-0170, p83. Rotterdam, the Netherlands, October 16–21, 2016.

Individual meetings are possible with industry actors interested in hosting the research team to present the major findings.

Financial contributions

Partnership for innovation in dairy production and dairy processing (EPI2011-2017):

- Fonds de recherche du Québec – Nature et technologies
- Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
- Novalait

The scholarship FAST from INAF under the CRSNG CREATE program was granted to Audrey Gilbert, covering the first two years of her Ph.D. when she was not yet eligible for funding by this project.

Total budget: $220,000

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Controlling the energy balance through milking rather than diet

Duration: 2013 – 2017

Highlights

- At the start of lactation, there is an imbalance between inputs and nutrient needs in dairy cows. This has a major negative impact on disease incidence (e.g., hyperketonemia, mastitis) and on reproductive performance.
- The conventional approach to controlling this imbalance consists of increasing the energy density of the ration at the beginning of lactation.
- An alternative approach would be to temporarily decrease nutrient needs through incomplete milking (10L/day) for the first 5 days of lactation.
- A randomized control trial was completed on 800 cows from 13 commercial dairy farms to evaluate the impact of the practice on the cows' energy balance and its consequences.
- Incomplete milking significantly reduced the blood concentration of ketones and hyperketonemia between the 4th and 17th days in lactation (DIL); cows following an incomplete milking protocol seem to adopt a desirable resting behaviour (i.e., time lying down) earlier in the lactation period. They have the same production levels as that of cows following a conventional milking protocol as of the second week of lactation. They are also better able to eliminate mammary gland infections and have better reproductive performance.

Objectives

The objective of the study is to measure the impact of incomplete milking of multiparous cows during the first week of lactation on:

- Serum concentrations of ketone bodies.
- Cows' comfort levels during the treatment period.
- The incidence of major metabolic and infectious diseases (hyperketonemia, clinical and sub-clinical mastitis, metritis and endometritis).
- Reproductive performance.
- Milk production.
- Culling rate.

Results and potential benefits

The results of this project help us confirm the usefulness of an innovative energy balance management method during the transitional period in a commercial context. Adjusted milking (10 to 14L/day between the 1st and 5th DIL) temporarily reduces milk production (approximate reduction of 50%) and the energy needs of multiparous cows during the transitional period, thereby significantly improving the negative energy balance and the ketonemia regularly observed in these cows. The levels of hyperketonemia were reduced by approximately 60% between the 4th and 7th DIL and by approximately 35% between the 8th and 17th DIL. Between the 8th and 17th DIL, the impact of incomplete milking on hyperketonemia levels was 2.4 times greater in cows in the second lactation than in older cows.

The cows' behaviour did not seem affected by incomplete milking, which suggests that incomplete milking does not cause discomfort. Furthermore, as of the 14th DIL, cows following an incomplete milking protocol had a similar production level to that of cows following a conventional milking protocol. An increase in the elimination rate of intra-mammary infections was observed in cows following an incomplete milking protocol, which suggests an improved immune system in these cows. However, the incidence of intra-mammary infections, clinical mastitis and uterine diseases was not significantly reduced. Finally, there was a very significant positive impact on fertility (time until successful breeding). Cows following an incomplete milking protocol are 24 times more likely to become pregnant during the 21 days following the end of the voluntary waiting period. Finally, incomplete milking did not affect the risk of culling.

A positive effect on the profitability of herds is expected, given the minimal costs and major positive impacts associated with this alternative management method during the transitional period. Lastly, the results of this research on multiparous cows will potentially help guide future research on the impact of this management method on the performance of primiparous cows.

Professionals trained

Pierre-Alexandre Morin (Masters), expertise in veterinary epidemiology; currently a veterinary practitioner at Université de Montréal's ambulatory clinic (Faculty of Medicine). Pierre-Alexandre plans to pursue clinical teaching in veterinary medicine.

Catarina Krug (PhD), expertise in epidemiology and animal behaviour; plans to continue her research on animal behaviour and health.

Ève-Marie Lavallée-Bourget (summer research internship), student in the Doctor of Veterinary Medicine (DVM) program.

Caroline Bergeron (summer research internship), student in the Doctor of Veterinary Medicine (DVM) program.

Josée Lemay-Courchesne (summer research internship), student in the Doctor of Veterinary Medicine (DVM) program.

For further information

The research results are already transferable to dairy farmers. An article on the project was published in the November 2017 issue of *Le producteur de lait québécois*. Moreover, a presentation was given at the 2017 Symposium sur les bovins laitiers. Other communication activities (articles, training sessions and conferences) are planned for users in collaboration with our partners at Valacta and as part of the transfer activities of the FRQ-NT Op+Lait strategic cluster (www.oplait.org) and Novalait.

Financial contributions

Partnership for innovation in dairy production and dairy processing (EPI2011-2017):

- Fonds de recherche du Québec – Nature et technologies
- Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
- Novalait

Total budget: $220,000

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Improving fodder grass in the context of climate change

Duration: 2014 – 2018

Highlights

- Due to climate change, fodder production in the province of Quebec must be adapted.
- Timothy grass is a plant that grows well in cool climates, but the current climate is beginning to make it less advantageous.
- An alternative must be found to timothy grass that is better suited to the growing conditions in Quebec.
- When mixed with alfalfa, tall fescue, meadow fescue and meadow bromegrass are associated with estimated milk production per hectare that is comparable to that of the alfalfa/timothy grass mix. These grasses thus represent possible alternatives to timothy grass throughout Quebec.
- Timothy grass remains an interesting grass in our current climate, as the yield and nutritional value of the alfalfa/timothy grass mix were among the highest when comparing different mixes.
- Tall fescue in the form of haylage or semi-dry silage can replace timothy grass in cow rations. Cows fed with rations consisting of timothy grass or tall fescue in the form of haylage had similar dietary intake, milk production and milk fat content.
- Cows fed rations consisting of tall fescue in the form of semi-dry silage consumed less but had similar milk production.
- When the dairy farm is considered as the only decision-making unit, the best grass/alfalfa binary association from a technical-economic and environemental point of view is currently being analyzed.
- The development of timothy grass germoplasms with the best regrowth rate is ongoing.
- Increased knowledge on the best forage species to grow will enable dairy farmers in Quebec to make more informed choices in the context of climate change.

Objectives

GENERAL OBJECTIVE: to determine the best fodder grass for production in the face of climate change.

SPECIFIC OBJECTIVES:

- Assess alternative grasses to timothy grass grown in association with alfalfa.
- Assess the impact of replacing timothy grass with tall fescue preserved in the form of wilted or semi-dry silage in the fodder rations of lactating cows.
- Assess the impact of the choice of pure plant species and alfalfa-based binary associations in crop rotations, with or without intensive management, on farm profitability.
- Develop a selection method and produce germoplasms that can be used to develop rapid regrowth varieties of timothy grass.

Results and potential benefits

The alfalfa/timothy grass mix performed well throughout Quebec in our current climate. The alfalfa/meadow fescue, alfalfa/tall fescue and alfalfa/meadow bromegrass mixes are possible alternatives to the alfalfa/timothy grass mix as they have comparable seasonal yields, their persistence is good in the first three years of production and the estimated milk production per hectare associated with these mixes is similar to that of the alfalfa/timothy grass mix. The alfalfa-based mixes including the festulolium and perennial rye grass varieties studied do not seem to be feasible alternatives to timothy grass in Quebec. Harvesting binary alfalfa-grass mixes at the early-bloom stage of alfalfa promotes the persistence of the mixes, their dry matter yield and the estimated milk production per hactare of fodder. There is some variability between the germoplasms of timothy grass around the world for regrowth leading to a second cut, such that genetic selection could be undertaken to develop rapid-regrowth varieties. Tall fescue in the form of haylage or semi-dry silage can be used to replace timothy grass in cow rations as milk production is similar no matter which of the two grasses the ration consists of. This study also helped us confirm interest in using alfalga/grass mixes in rations. The results are currently integrated in a farm management computer model and will help us to determine the economic and environmental impacts, on farm as a whole, of the practices that will b e proposed as part of the project.

Professionals trained

Florence Pomerleau-Lacasse is the master's student at McGill University who completed the fodder grass plot testing component from 2015 to 2017.

Anne-Marie Richard is the master's student at Université Laval who was in charge of the animal testing component comparing the use of tall fescue to that of timothy grass in dairy cow feed.

Véronique Ouellet is currently completing her PhD in animal science, and Jean-Philippe Laroche is currently finishing his bachelor's degree in agronomy (distinction profile) at Université Laval. They are working on the modelling component of the project. The results will help us to determine the economic and environmental impacts, on the farm as a whole, of the practices that will be proposed as part of the project.

For further information

The results of this project will be communicated in popular science articles, and posters at the Symposium des bovins laitiers and the Forum Techno Novalait and related science information days, as well as through presentations.

Financial contributions

Partnership for innovation in dairy production and dairy processing (EPI:2011-2017):

- Fonds de recherche du Québec – Nature et technologies
- Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
- Novalait

Centre de recherche en sciences animales de Deschambault for animal experimentation

AAFC for germoplasm selection

Total budget: $190,000

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Alain Fournier
Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec

Robert Berthiaume
Valacta

Industrial research chair in efficiency of milk processing

Duration: 2014 – 2019

Highlights

- The dairy processing sector is currently developing an innovative industrial approach to optimize the energy efficiency of the processes used.
- Membrane separation technologies have been identified as a research model due to their widespread use in the processing chain for milk and its co-products. These processes are likely to have a positive and significant effect on reductions in production costs (purchasing and replacing membranes), the use of natural resources (water, energy) and the generation of waste (sweet and acidic whey, wash water, etc.) that must be treated.
- Research conducted by the NSERC-Novalait Chair on Process Efficiency in Dairy Technology aims to optimize the use of natural dairy components while improving membrane process efficiency. These improvements will allow for the development of innovative solutions that can be applied in the short term in the dairy processing industry.
- The research has focused on process improvement with regard to energy efficiency. The experimental approach for each of these aspects of the project involves an **impact evaluation** as well as a **characterization of the state of the system's constituents**, which allows for the development of hypotheses concerning the potential points to be improved upon in regards to process efficiency.

Objectives

The research completed by the Chair aims to:

- Identify new approaches that will improve the use of milk's natural components while minimizing the environmental impact of processes (water, energy, losses/waste).
- Develop measuring tools and the appropriate inventory methods in order to be able to quantify the effects of processes on energy efficiency parameters for dairy processing.
- Apply the new tools to problems concerning the processing of milk and its co-products.

Results and potential benefits

Economic benefits

- **Increased productivity**: the contribution of filtration parameters on the appearance of clogging and the increased energy consumption of baromembrane systems during the fractioning of dairy components helps generate useful data that can be used as a decision-making tool for dairy processors in order to optimize their plant's efficiency.
- **Reduced processing costs**: the study of biofilm establishment mechanisms, their contribution to performance losses in baromembrane processes and the implementation of solutions that help control their long-term formation will help dairy processors to minimize costs related to the replacement and cleaning of membranes.
- **Improved quality of end products**: understanding mechanisms related to modifications in the physico-chemical properties of concentrated dairy matrices helps optimize their stability and quality during storage. As a result, this increases their potential for use in cheesemaking.

Environmental benefits

All of the studies related to optimizing filtration parameters in order to control organic and microbiological clogging leads to 1) **a reduction in inputs and outputs** related to a reduction in energy consumption and cleaning solutions/effluents, as well as optimization of the lifespan of membranes, and allow for 2) **the development of energy efficiency simulation software** adapted to dairy processes, making it possible to test different production scenarios related to economic data (cost of raw materials and resources) as well as environmental data and to choose the most energy efficient option.

Social benefits

As consumers are becoming increasingly socially responsible in their consumption habits, all of the advantages presented above will help to better meet the needs of consumers, in the long-term, from a sustainable development point of view.

Professionals trained

Outgoing researchers working under the Chair are all trained experts with a specific interest in dairy science, process efficiency and sustainable development.

Daniel Tremblay-Marchand (MSc) – January 2016*
Stéphanie Méthot-Hains (MSc) – April 2016*
Dany Mercier-Bouchard (MSc) – December 2016
Camile Gavazzi-April (MSc) – April 2017
Gabrielle Beaulieu-Carbonneau (MSc) – April 2017
Iris Dussault-Chouinard (MSc) – September 2018
Isabelle Fournier (MSc) – December 2018
Virginie Damphousse (MSc) – December 2018
Amandine Bouyer (MSc) – December 2018
Scott Benoit (PhD) – August 2017
Agathe Lauzin (PhD) – April 2018
Julien Chamberland (PhD) – April 2018

* Diploma obtained

For further information

Group members will use a range of means of communications available to reach enterprises who will be able to put the results of this research into application. The results will be presented at conferences (STELA Colloquium, American Dairy Science Association, IDF Symposium, Forum Techno Novalait) and published in scientific journals. The knowledge transfer tools available through Novalait, STELA (INAF) and the chair's website will also be leveraged.

Financial contributions

National Sciences and Engineering Research Council of Canada

Novalait

Canadian Foundation for Innovation (Leadership funds)

Université Laval

Total budget: $1,775,000

Contact persons

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École Polytechnique de Montréal

Michel Britten
Agriculture and AgriFood Canada

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.
- Two bioprotective and biocompatible lactic acid bacterial consortia that could be used to produce and conserve Cheddar cheese were developed.
- Natural ingredients with synergistic antimicrobial compounds produced by bioprotective lactic cultures were also developed.
- Technological processes were developed for the production and long-term stabilization of bioprotective cultures and functional ingredients.
- Capsules and hydrogels with antimicrobial activities for conserving fermented dairy products or cleaning work surfaces and equipment were developed.
- These results will constitute the first proof-of-concept of the application of natural bioactive compounds in food production and processing.

Objectives

The general objective is to develop knowledge and expertise relating to the isolation, identification and characterization of lactic acid bacteria exhibiting antimicrobial activity with a high potential for use as a bioprotective agent. 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. 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 yogourt 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.

Professionals trained

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

Laurent Dallaire and **Léo Daviaud** completed their master's project as part of the chair. Additionally, a master's student and four PhD students are currently working on their research projects:

Hélène Pilote-Fortin, master student

Liya Zhang, doctoral student

Hebatoallah Hassan, doctoral student

Samira Soltani, doctoral student

Sabrine Naïmi, doctoral student

For further information

- Presentation of the results at Novalait's Forum Techno and annual meeting as well as Agropur's annual scientific meeting.
- 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.*
- Patenting.
- Development of a spin-off (Laboratoire Innodal) by a chair student for the production of large-scale bioprotective cultures.
- Tests aiming to scale up reuterin production were completed. The scaling up is scheduled for 2019.
- 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 Consortium de recherche et innovations en bioprocédés industriels au Québec (CRIBIQ).

Industrial Partners are:

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

Total budget: $1,916,685

Contact persons

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Industrial Research Chair on the Sustainable Life of Dairy Cattle

Duration: 2016 – 2021

Highlights

- Dairy farmers are tasked with challenge of providing the market with high quality products while ensuring an increased productivity by understanding the nutritional and physiological needs of the cow, and integrating the social expectations regarding the environment, animal welfare, and economical accessibility.
- Increasing the lifespan of dairy cattle raised in a comfortable and healthy environment is a sustainable solution to decrease involuntary culling and increase the profit of the enterprise, while limiting the environmental footprint and answering the concerns of animal welfare.
- On these premises and in order to have a new expertise in this area, the industrial research Chair on the sustainable life of dairy cattle took form at McGill University on January 1st, 2016.This important research initiative aims to deliver concrete data and tools to help dairy farmers optimize comfort, with a particular focus on tie stall farms, and increasing the lifespan of cows.
- The research Chair also aims to improve the recommendations of welfare to help prepare farmers for the implementation of the welfare component of the proAction® national program.
- The work from the past two first years produced results regarding the modifications on the configuration of stalls to improve the movement opportunities and comfort of dairy cows.

Objectives

The research Chair's objective is to optimise the comfort, with a particular focus on tie stall farms, and to increase the lifetime of cows while considering the sustainability of dairy farms.

Results and potential benefits

The Chair pursues its research activities based on three main themes. Here are the results of the two first years:

Theme 1: Cow comfort and herd management

- New knowledge on behavioural measures, which could be used to automate the monitoring and detection of welfare problems in tie stalls, have been developed. For example:
  - A 3-D pedometer (IceTag) precisely measures the numbers of steps taken by a cow in her stall (Shepley et coll. 2017 Agriculture 7:53).
- Many studies have been conducted to evaluate the impact of the stall configuration on the opportunities of movement and cow comfort, here is what we found:
  - We tested different height-forward combinations of the tie rail position following the cow neck line: the neck injuries move with the bar. Next step: what alternatives? (for more information, consult the summary of the student Jessica St John from the Forum 2018).
  - The cows with a longer chain seem more comfortable with their environment: they will hesitate less when lying down, while the cows in a wider stall will display better lying postures and will utilise the additional space they are provided with to extend their legs (for more information, consult the summary of the student Véronique Boyer from the Forum 2018).

Theme 2: Cow Longevity (results coming)

- The second research theme will look at long-term profitability and animal survival measures in herds. The replacement animals and the cows in production will be examined. The projects under this theme will benefit from combined data collected on commercial farms and databases from Valacta.

Results and potential benefits, continue...

Theme 3: Environment and society (results coming)

- The third research theme will allow us to widen the perspective on the international standards, the studies on life cycle, and the acceptability of consumers. This will involve validating that the measures to improve welfare and longevity are in accordance with the global sustainability (environmental, economical and social) of dairy farms.

Professionals trained

The research chair is involved in training highly qualified personal. Since its creation, 31 people have been trained. From 2016-2017: one Ph.D. student, five thesis based masters students and seven applied masters students (not thesis based), Four postdoctoral fellows, four research assistants, nine bachelors students and one research exchange student are actively involved.

Elise Shepley – Ph.D.

Masters thesis: **Maria Puerto Rodriguez**, **Sarah McPherson**, **Erika Edwards** (in collaboration with University of Tennessee, USA), **Véronique Boyer**, **Jessica St John**

Applied masters: **Maria Francesca Guiso** (University of Sassari, Italie), **Giovanni Obinu** (University of Sassari, Italie), **Manon Demaret** (ISA Lille, France), **Géraud Plas-Debecker** (AgroCampus Ouest, France), **Marianne Berthelot** (AgroCampus Ouest, France), **Anthony Pic** (vetAgroSup Clermont, France), **Sirine El Hamdaoui** (ISA Lille, France)

Postdoctoral fellows (in collaboration with Valacta): **Maxime Leduc**, **Daniel Warner**, **Liliana Fadul Pacheco**, **Hector Delgado**

For further information

- Two articles following the work achieved by the chair were published in the magazine *Le producteur de lait québécois*.
- Poster presentations at the Forum Techno Novalait de 2018
  http://www.nserc-crsng.gc.ca/Chairholders-TitulairesDeChaire/Chairholder-Titulaire_fra.asp?pid=954
- Chair holders' website: https://www.mcgill.ca/animal/staff/elsa-vasseur
  http://www.nserc-crsng.gc.ca/Chairholders-TitulairesDeChaire/Chairholder-Titulaire_fra.asp?pid=954
- The Chairs Blog: http://cowlifemcgill.blogspot.ca/
- The Chairs twitter account: @CowLifeMcGill

Financial contributions

The research chair is funded through the Industrial Research Chair of Natural Sciences and Engineering Research Council of Canada (NSERC).

The industrial partners are:

- Novalait
- Dairy Farmers of Canada
- Valacta

McGill University has also contributed financially.

Total budget: $1,720,000

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Valacta Team

How silage and raw milk affect dairy product quality

Duration: 2013 – 2017

Highlights

- Raw milk also presents an environment that can be contaminated by thermo-resistant flora and spores from fodder and indigenous flora.
- The microbiological quality of milk is therefore a major commercial issue and a constant technological challenge both on farms and in plants.
- Silage is humid fodder that is preserved through the addition of lactic acid bacteria inoculants.
- These aromatic lactic acid bacteria can have an undesirable impact on milk acidification and on the organoleptic quality of processed dairy products.
- Twenty-four farms divided into five groups that are representative of the primary cow feeding methods were sampled two times to determine the prevalence and diversity of the microbiota of silage and raw milk.
- The lactic acid bacteria selected from 1,400 isolates from silage and raw milk from the 24 farms will be tested for their resistance to heat treatment and their contribution to the production of volatile compounds during the production and ageing of cheddar cheese.
- The anticipated results will help farmers identify the best silage management practices in order to optimize the microbiological quality of milk and help processors control the sources of microbial contaminants.
- While *Lactobacillus buchneri* is prevalent in both inoculated and uninoculated silage, the species is rarely found in raw milk.
- Two of the strains isolated from raw milk–identified as *Lactobacillus casei* and *Lactobacillus plantarum*–were selected for their thermoresistance. In the Pearce test (Cheddar cheese production), these strains did not have an impact on the acidification kinetics of *Lactoccus lactis* SK11starter culture; however, in a curd model, they were able produce volatile components during the ripening stage.

Objectives

- GENERAL OBJECTIVE: Determine the prevalence and diversity of the microbiota of silage and raw milk and their impacts on the organoleptic quality of dairy products.
- HYPOTHESIS 1: Estimating the diversity and prevalence of bacteria will allow us to know if the use of lactic acid bacteria inoculants in silage is responsible for the presence of indigenous flora that alter milk.
- HYPOTHESIS 2: Aromatic lactic acid bacteria that are resistant to heat treatment are responsible for the production of undesirable volatile compounds in cheeses.

Results and potential benefits

In terms of new knowledge:

Impact of different types of silage in a cow's diet on the microbiological quality of raw milk:

- Overall, 226 bacterial taxons and 648 fungal taxons were identified using metataxonomics.
- The bacterial species found in the milk were also present in the silage and hay at a proportion of approximately 50% to 80% and approximately 60% to 70% for the fungal species.
- While *Lactobacillus buchneri*, is prevalent in both inoculated and uninoculated silage, the species is rarely found in raw milk.

Effects of milk's indigenous flora on the organoleptic properties of processed dairy products:

- Two of the strains isolated from raw milk–identified as *Lactobacillus casei* and *Lactobacillus plantarum*–were selected for their thermoresistance. In the Pearce test (Cheddar cheese production), these strains did not have an impact on the acidification kinetics of *Lactoccus lactis* SK11starter culture; however, in a curd model, they were able produce volatile components during the ripening stage.
- This link between the activity of the secondary flora and the profile of the volatile components in Cheddar cheese will be evaluated using transcriptome sequencing.

Results and potential benefits, continue...

Potential Benefits

- Optimization of the microbiological quality of milk used in cheesemaking.
- Control of the organoleptic quality of dairy products.
- Reduction in numbers of rejections of poor-quality processed dairy products.
- Maintain of quality standards for milk and dairy products.

Professionals trained

Mérilie Gagnon (PhD): Phenotypic and genotypic characterization of isolated lactic acid bacteria in silage and raw milk: antibacterial activity, thermoresistance and the production of volatile compounds. Mérilie Gagnon has acquired expertise in: i) isolating and identifying lactic acid bacteria in different types of fodder and in raw milk and ii) screening the isolates for different metabolic characteristics. She would like to pursue a career in research in the field of microbiology, in association with dairy products.

Alexandre Jules Kennang Ouamba (PhD): Comparative analysis of the prevalence and phylogenetic structure of microbial communities in silage and cow milk. Alexandre is developing expertise in: i) the metagenomic analysis of microbial flora (bacteria, yeasts and moulds) of silage and raw milk through high-output multiplex sequencing and MARISA and PMA-qPCR techniques; and ii) the statistical analysis of metagenomic data (multivariate analyses, ordering, co-occurrence/co-exclusion networks). He is interested in research in food microbiology and related fields.

For further information

The main project contributions were made available to dairy farmers in the articles published in *Le Producteur de lait québécois* and *La Terre de chez nous*. During the project, results were communicated through presentations and poster sessions at the general assembly of the Op+LAIT strategic cluster and at the Novalait Forum Techno. In 2018, the most salient results will be presented at the Cheese Symposium (Rennes, France) and the Food Microbiology (Berlin) international conferences.

Financial contributions

Partnership for innovation in dairy production and dairy processing (EPI2011-2017):

- Fonds de recherche du Québec – Nature et technologies
- Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
- Novalait

Canada Research Chair on Lactic Cultures Biotechnology for Dairy and Probiotic Industries

Total budget: $220,000

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ONGOING PROJECTS

2018

Novalait

Improving eco-efficiency in milk processing by optimizing the usage of milk components: the case of Greek yogurt

Duration: 2014 – 2018

Highlights

- Concentrated "Greek-type" yogurt has seen an exponential increase in consumption but has consequently generated acidic whey that is managed as residual matter.
- This environmental problem, the costs of development related to Greek yogurt production and its value to consumers raises questions about eco- efficiency (EE) in milk processing.
- This project allowed for a more specific comparison between the environmental impact (using a life cycle assessment (LCA) approach) of ultrafiltration before filtration (UF-MILK) and post fermentation (UF-YOG).
- The ultimate goal of the research is to develop decision-making tools for industrial dairy processors, based on life cycle assessment data and the notion of industrial ecology, in order to optimize the use of milk's natural components.

Objectives

- Describe the impact of technological choices on process efficiency and on the use of milk components for a model Greek yogurt production sequence.
- Develop an analysis framework to assess the EE of the processing of Greek yogurt.
- Identify and assess the external channels for the valorization of co-products and industrial synergies.
- Develop and validate a modelling tool for environmental and economic impact assessments and an optimization tool based on the EE indicator for milk processing.

Results and potential benefits

Economic benefits

- **Increased productivity**: The experimental data generated on a pilot scale show that choosing the milk concentration process before fermentation (UF-MILK) allows for a better usage of milk components than if ultrafiltration is completed after the fermentation stage (UF-YOG). The absence of lactic acid in the UF-MILK co-product facilitates valorization of the milk solids present in the permeate.
- **Reduced production costs**: Volumic concentration factor used to concentrate milk has a direct effect on production costs as well as on the environmental impact of Greek yogurt. Moreover, a mapping of technological scenarios for Greek yogurt production (centrifugation vs. ultrafiltration) and of the channels for the valorization of co-products allowed us to develop simulations of matter and energy balances applicable to each scenario in Quebec's industrial context.

Environmental benefits

- As milk production is responsible for approximately 80% of the impacts on the life cycle of Greek yogurt, a better use of processed milk components will create significant environmental benefits.
- The work will allow us to develop an eco-efficient methodology adapted to the specific characteristics of the dairy industry.

Social benefits

By optimizing the use of organic matter in the Greek yogurt value chain, the knowledge gained as part of this project will allow players in the industry to generate less waste and more value (principles of circular economy). This work addresses consumer concerns from a sustainable development point of view.

Professionals trained

Adriana Paredes-Valencia (MSc)
Holding a graduate diploma in sustainable development (France), Adriana has acquired practical experience in dairy research where she has utilized life cycle assessment (LCA) fundamentals in order to interpret her own data. Her professional interests are directly related to sustainable development in the food processing sector.

Catherine Houssard (PhD)
With a background in agri-food engineering, Catherine has worked in the industrial sector for over 15 years. Catherine has begun PhD research on the concept of energy efficiency in order to better adapt energy usage to the dairy industry. Her objective is to help develop new skills in energy efficiency and the operationalization of sustainable development concepts in the agri-food industry.

For further information

Group members will use different means of communication to reach enterprises that will be able to put the results of this research into application. The results will be presented at conferences (STELA Colloquium, Forum Techno, American Dairy Science Association, IDF Symposium) and published in scientific journals. The knowledge transfer tools available through Novalait, STELA (INAF) and the NSERC-Novalait chair's website will also be leveraged.

Financial contributions

Partnership for innovation in dairy production and dairy processing (EPI 2011-2017):

- Fonds de recherche du Québec – Nature et technologies
- Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
- Novalait

Total budget: $190,000

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Systems biology applied to cheddar production

Duration: 2014 – 2018

Highlights

- One of the challenges in the milk processing industry is producing high quality cheeses on a consistent basis.
- Many factors influence quality, including the microbiological composition of milk and the effectiveness of starter cultures and bacteriophages.
- This project investigates these factors using a "systems biology" approach to better understand the impact of the microbiological network on cheddar production.
- Systems biology integrates different levels of information to develop an operating model for the entire system.
- Systems biology uses techniques to quantify changes in the genome, transcriptome, proteome and metabolome in response to a given situation (cheddar, in this case).
- This ambitious project will generate new results that will put dairy processors in a very competitive position internationally.
- Functional assembly (from the genome to the metabolome) will also provide added-value in order to better understand and intervene in regards to the major variables affecting these products.

Objectives

- OBJECTIVE 1 - Determine the microbiome and virome of milk and cheddar.
- OBJECTIVE 2 - Determine the microbial and viral transcriptome of milk and cheddar.
- OBJECTIVE 3 - Determine the microbial and viral proteome of milk and cheddar.
- OBJECTIVE 4 - Determine the metabolome of milk and cheddar.
- OBJECTIVE 5 - Determine the biology of the cheddar system. This inclusive objective will allow us to pin-point trends during the ageing process for cheddar. A mathematical algorithm which will use all of the information produced (OBJECTIVES 1 to 4) will be developed to calculate divergence between the samples.

Results and potential benefits

- OBJECTIVE 1: Various batches of Cheddar cheese are monitored after the first day of production and then at various intervals. A protocol has been adapted to separate bacteria and phages from milk matrices. A number of new *Lactococcus lactis* genomes (draft) are now available. We have also built a database containing sequences of DNA from microbial species known to be part of the microbiome of various cheeses. The microbiome of approximately 60 cheeses is already available.
- OBJECTIVE 2: We have developed a protocol for isolating RNA from dairy samples (milk, cheese).
- OBJECTIVE 3: Using different proteomic approaches and a *Lactococcus lactis* bacteriophage model system, we were able to detect 78% (38/50) of the phage proteins and 56% (1,332/2,383) of the bacterial proteins. We have identified 209 *L. lactis* proteins that are uniquely expressed during infection through p2 phage.
- OBJECTIVE 4: We have developed a protocol for extracting and analyzing non-volatile metabolites and lipids contained in cheese using LC-MS-MS. We have generated over 750GB of data for approximately 60 cheeses. Our preliminary tests have showed great metabolic and lipid diversity in the analyzed samples. We have observed both increases and decreases in ions based on the ageing stage of the cheese. The identified metabolites could serve as a marker of quality and be linked with certain organoleptic properties.
- OBJECTIVE 5: This last integrating objective is not yet completed. Ultimately, we will propose a mathematical algorithm that will integrate all of the data. It will allow us to pinpoint trends during the cheese ageing process and identify specific indicators of cheddar quality. Ultimately, this approach will create a network which will allow for interpretation of the microbial and viral composition of cheddar. It will also provide new insights on how to modulate the ageing process in order to improve consistency and quality.

Professionals trained

Marie-Laurence Lemay, PhD student in microbiology

Pier-Luc Plante, PhD student in bioinformatics

Alexia Lacelle-Côté, master's student in microbiology

Frédéric Raymond, post-doctoral researcher in bioinformatics

Simon Labrie, post-doctoral researcher in microbiology

Jessie Bélanger, undergraduate student in microbiology

For further information

Information will primarily be distributed in two ways:

- The knowledge acquired, as well as a user-friendly bioinformatic tool, will be made directly available to dairy farmers through meetings with members of the Novalait steering committee and during the Novalait Techno Forum.
- We also plan to publish our results in respected international journals with the review committee (for example, Appl. Environ. Microbiol.).

Financial contributions

Partnership for innovation in dairy production and dairy processing (EPI2011-2017):

- Fonds de recherche du Québec – Nature et technologies
- Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
- Novalait

The industrial partner has provided cheese and lactic ferments.

Total budget: $189,926

Contact persons

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Searching the microflora of local milks and cheeses

Duration: 2016 – 2019

Highlights

- Cheese microflora is complex and contains microorganisms used as cultures, as well as microorganisms found in the milk's natural microflora and ripening rooms.
- Little is known about the indigenous microflora found in Quebec fine cheeses and about the contribution of its different species to the production of aromatic compounds.
- This project aims to use new metagenomic methods (metabarcoding) to identify species of bacteria, yeasts and moulds that contribute to the ripening of Quebec cheeses.
- As part of the project, we aim to create an inventory of natural species found in 17 farm-house cheeses from Quebec and report on local cheese typicity.
- Strains from local cheeses are in the process of being sequenced so that we may identify their activities based on their metabolic pathways.
- This project will help to control the microflora of Quebec cheeses and enable us to improve consistency in the cheeses' sensory properties.

Objectives

To identify the microflora resulting from the ripening of a number of fine cheeses from Quebec using genomics; to understand the roles and activities of typical species of bacteria, yeasts and moulds in order to predict the production of aromatic compounds which give taste and flavour to Quebec cheeses.

More specifically:

- OBJECTIVE 1 - To complete an inventory of the species present in fine cheeses from Quebec.
- OBJECTIVE 2 - To identify the metabolic pathways of keys species from the secondary microflora of fine cheeses.
- OBJECTIVE 3 - To detect changes induced by the different ripening parameters on the expression of these metabolic pathways.

Results and potential benefits

The inventorying of natural species of Quebec fine cheeses will allow us to determine the impact of the region on cheese microflora and to report on local cheese typicity. To this end, a metabarcoding method has been optimized for analyzing cheese using universal genetic markers for mycetes (ITS) and bacteria (rDNA 16S). The method is now optimized and can be used. To evaluate the constancy of cheese microflora, seveteen (17) cheeses were evaluated on two occasions: once in 2015 and again in 2018.

The study will then focus on the intrinsic characteristics of local milk and processing parameters (heating of milk, cow breeds, etc.) that have an impact on the growth of microorganisms during ripening. The project will assess their importance and impact within cheese ecosystems.

Furthermore, the project aims to better understand the metabolic potential of 12 bacteria and 5 isolated yeasts from the cheeses' secondary microflora. Comparative genomics and bioinformatics will help predict and characterize their metabolic pathways to determine their capacities to produce aroma and flavour precursors. The genomic characterization of these strains will shed light on their roles in the development of the organoleptic characteristics associated with the ripening of fine cheeses.

It is expected that identifying these species will highlight the specific microbiological characteristics of local cheeses. Knowledge about these species will allow us to better control them, will serve as an element of distinction for obtaining value-added terms and will help improve consistency in the products.

Professionals trained

3 masters students:

- **Andréanne Lamarche**: Detection of indigenous microorganisms
- **Annick Raymond-Fleury**: Cheese metagenomics
- **Gabrielle Jacquemet**: Identification of metabolic pathways and the impact of different production parameters

For further information

- Cheese factories participating in this project will receive a confidential report on the characteristics of their cheese microflora. The report will describe the specific microorganisms identified in the cheeses and compare these ecosystems with the average of the analyzed cheeses.
- Moreover, the results will be published in scientific articles and presented at conferences, including the STELA symposium and Novalait's Forum Techno. Direct discussions with processors will also be held. Finally, popular science articles could be published, and segments in popular science television programs could be aired.

Financial contributions

Partnership for innovation in dairy production and dairy processing (EPI2015-2019):

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

Total budget: $283,245

Contact persons

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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.
- In this project, we propose developing a genomic analysis tool that would leverage the genetic variability present in the Holstein species to diversify specific regions of the genome known to be involved in health and fertility traits. The objective is to improve these traits that respond poorly to current selection processes by drawing from the species' existing genetic diversity.

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:

- Identify the regions known to influence the health and fertility of dairy cattle.
- Choose targets.
- Develop a genome analysis too.
- Program the interface to make it easy to use.
- 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 species 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. So far, more than 99,652 regions of the genome that are known to be involved in bovine biological functions have been identified in the literature and correspond to 574 different traits. This information was sorted and structured in a database. As part of this project, 177 bulls from four genetics companies as well as 10 cows were genotyped. The genetic diversity for the targeted regions was calculated for each animal, and correlation values were calculated based on the genetic values associated with the different traits considered by the national genetic improvement program. The correlation values are high (0.34 to 0.51) for health and fertility traits and are low for production traits, suggesting that the targeted regions are not involved in these traits. A validation phase is ongoing using DNA from 200 cows that were shown to be healthy or susceptible to various health and fertility disorders. The ultimate objective is to improve health and fertility traits in these animals, which will improve their longevity and therefore reduce remplacement costs.

Professionals trained

The project will allow for the training of:

• a master's student (**Alexandra Carrier**) who will be involved in the programming of the tool.

• a research professional (**Alexandre Bastien**) who will also be involved part-time in the programming and to assist the student.

Ultimately, these individuals will develop new skills in genomic analysis. These skills are greatly needed in Quebec if we would like to remain competitive in the field of genetic selection (all species combined).

For further information

This is an applied research project whose target public is mainly actors involved in genetic selection and dairy farmers. We will present our results at the Quebec Dairy Cattle Symposium and the Novalait Technological Forum, and we will produce an article for a journal read by dairy farmers (most likely Le producteur de lait québécois).

Financial contributions

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

• Consortium de recherche et innovation en bioprocédés industriels au Québec

• Fonds de recherche du Québec – Nature et technologies

• Novalait

Total budget: $186,706

Contact persons

Project supervisor:

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Putting back to day 120 the insemination of cows in metabolic stress: an idea to validate for the health and profitability of the herds

Duration: 2016 – 2019

Highlights

- This project brings together complementary genomic and economic expertise applied to dairy cows.
- Université Laval and the University of Wisconsin will partner with Valacta to document an emerging phenomenon: intergenerational epigenetic programming and its technico-economic impact for farmers.
- The metabolic status of mother cows may have an impact on the metabolic programming of the next generation in addition to traditional genes.
- Cows with an energy deficit at the time of insemination may potentially have female offspring that are less efficient at producing milk.
- The project examines this phenomenon under two aspects: 1) by completing a biological demonstration that the embryos carry remnants of this epigenetic programming, allowing us to measure the impacts and 2) by completing an in-depth economic analysis using a management tool that aims to identify the best time for insemination. This tool will be adapted to Quebec and will address this issue of energy deficit.

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

For the epigenetic portion of the project, it is possible that the embryos will be different between days 60 and 120, particularly for cows with a high BHB level. This distinction will allow us to choose markers associated with the metabolic status. Because the markers identified on the embryos will also be measured on post-natal tissues in order to identify the metabolic programming indicators, these markers will be validated on hundreds of individuals in order to obtain the strength necessary to develop a diagnostic tool. These indicators could be used for a range of purposes, including herd monitoring to choose the heifers to be retained and in conjunction with a tool for Boviteq to modulate the relative value of embryos and animals produced according to the mother's production. These tools are essential to the development of factual management practices, based on measurements taken at birth (biomarkers) and in milk (BHB), and quickly adopted by farmers. Ninety percent of the data for cows has been obtained, and the preliminary analysis of the data confirms numerous differences based on the cow's metabolic status. Thus seven days after fertilization, the cows, which are hungry, have different embryos. Now it's a matter of translating these differences into functionality and seeing if the calves retain the main markers discovered in the embryos.

For the economic portion of the project, a modification in the time of reproduction may have negative economic impacts on the current and future lactation of cows. There is a potential to save money if we are successful in identifying cows using the tool. The tools are in place to process Valacta's data, and the analysis has begun. The tool, which will be developed as part of this project, will then be made available to potential users with the goal of guiding farmers. The results of the project will therefore provide a new rating system that can be integrated in the service offer available to farmers by their centre of expertise (Valacta).

Professionals trained

Catherine Chaput: Master's student at Université Laval. Expertise in the genomic analysis of bovine embryos and the molecular impacts of BHB blood levels at the time of insemination.

Catherine Couture: Master's student at Université Laval in charge of the economic analysis of delaying insemination. Expertise in analyzing economic factors in dairy production.

For further information

We will present our results at the Quebec Dairy Cattle Symposium and the Novalait Techno Forum, and we will produce an article for a journal read by dairy farmers (most likely Le producteur de lait québécois).

Financial contributions

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

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

Total budget: $282,866

Contact persons

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

Duration: 2017–2020

Highlights

- There is a growing interest in using recycled manure as bedding for dairy cattle due to the rising costs of quality bedding.
- Using recycled manure has some drawbacks. Most notably, the bedding may serve as a reservoir for or support the growth of pathogens for cows and/or zoonotic pathogens.
- The risk of encountering these problems certainly varies based on the preparation method used and the bedding management practices in place.
- Currently, very little scientific information exists to make recommendations on best practices for producing and managing this type of bedding.
- This research project aims to develop recommendations for producing and safely using recycled manure bedding.
- The material and data required to achieve the objectives will all be obtained from 29 dairy herds using recycled manure bedding (RMB) and 63 herds using straw bedding.

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

We believe that the methods of completely transforming manure into compost will allow us to produce bedding with an acceptable level of risk. The potential for the various types of RMB to support the subsequent growth of bacteria remains to be established, as these are rich microbial ecosystems and therefore sites of intense inter-microbial competition.

Our assumption is that in certain conditions, these competitions are detrimental to the bacterial populations that we want to control (pathogens for cows and humans). Finally, our hypothesis is that mammary gland health and the milk bacteria count will be similar to that of herds using conventional bedding. However, the milk microbiota will probably differ because it may be moderately influenced by the milk environment during milking.

Ninety-two (92) dairy farmers have agreed to participate in this study, and sampling is currently underway. We have already observed the presence of certain zoonotic parasites and/or parasites that could harm the health of replacement cows in some certain unused RMBs (e.g., *Cryptosporidium* spp and *Coccidium*). Using RMB with young animals could therefore pose a risk to their health. The risk to human health remains to be analyzed.

The project will establish best practices regarding RMB production methods that will support dairy farmers who wish to safely integrate this bioprocess into their nutritional management strategy. The project will also inform dairy processors about the quality of milk produced through different methods. As a result, the project will make it possible to quantify the risk to animal and human health and the impact on milk quality.

Professionals trained

Annie Fréchette (masters), 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 (masters), expertise in parasitology at Université de Montréal.

Jessica Beauchemin (masters), 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 Valacta 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

Contact persons

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

Duration: 2017 – 2020

Highlights

- As of yet, no research carried out around the world has managed to develop a biomarker that would allow one to easily detect, from a simple milk sample, the health and welfare status of a dairy cow.
- The rationale of the current research project is that:
  - Research on animal welfare has led to the development of reliable behavioral indicators, ranging from clinical signs to measures 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.
- Recently, milk BHBA was found to be positively correlated with circulating BHBA (research by Denis-Robichaud et al. 2014. JDS 97:3314), indicating that milk sample analysis could be used to measure biomarkers of dairy cow health.
- The objective of this project is to develop precise indicators of dairy cow health and welfare that could be measured routinely in milk samples from individual cows or from herds.
- In the context of this project, physiological indicators will be analyzed along with the behavioral data collected during the experiments led by the chair on the sustainable life of dairy cattle.

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 within the first theme (Cow comfort and herd management) of the Research Chair on the Sustainable life of dairy cattle program, **by adding physiological data to the measures taken, among which most others are behavioral**.

4 experiments have been conducted on the Macdonald Campus Farm of McGill University, over a period of 36 months. These experiments aimed to evaluate the impact of stall configuration on the comfort and movement opportunities of tie-stall dairy cows. 4 aspects were evaluated:

1. position of the tie-rail;
2. chain length;
3. stall width;
4. stall length-manger wall height combination (currently running).

For more information, read the abstracts and posters produced by Jessica St John & Véronique Boyer, two of our students)

Behavioural measures 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.

Results and potential benefits, continue...

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 measures were selected according to the following four secondary objectives:

1. **Identify and evaluate the link between various indicators of health and welfare**
   - Plasma, milk and behavior 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 the cow in her tie-stall
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 of cows or herds using the same type of spectroscopy as what is currently used for milk recording**
   - FTIR spectroscopy

The collection and analysis of physiological data, in combination with behavioral data, is ongoing.

Professionals trained

Behavioral Measures Component: - 4 thesis-based Master students
Sarah McPherson
Erika Edwards (in collaboration with University of Tennessee, USA)
Véronique Boyer
Jessica St John

The work of these students is carried out as a part of the Chair on the Sustainable Life of Dairy Cattle. The results will be shared with the following two students:

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

1 Bachelor of Science Student (with distinction):
Shannon Manley

For further information

- An article featuring the project can be found in the June 2017 edition of the magazine *Le producteur de lait québécois*
- Website of the project manager: https://www.mcgill.ca/animal/staff/elsa-vasseur
- Blog: http://cowlifemcgill.blogspot.ca/
- Twitter account: @CowLifeMcGill

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

Total budget: $261,404

Contact persons

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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 Valacta'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 Valacta, 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:

- OBJECTIF 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.
- OBJECTIF 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 will take place on 11 commercial farms in the Lac St Jean region and will allow 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 will be analyzed, for a total of 3,000 samples (two milkings per day and a composite sample of these two milkings). IR analyses will be performed in Valacta's laboratories, and the method will be validated through gas chromatography (GC) 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. Valacta 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.

Professionals trained

- A master's student will be trained in the Animal Science Department at Université Laval.
- The student will gain expertise on the methods of measuring rumen pH in dairy cattle (rumen pH readers, FA profile through GC and FA profile through IR spectroscopy), on the interpretation of rumen pH measurements and on the causes of sub-acute ruminal acidosis and ways to prevent this disease.
- The student will develop his or her ability to work collaboratively with a research team and dairy farmers.

For further information

If the results obtained are conclusive, the acidosis test could be part of Valacta'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 Valacta.

Financial contributions

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POSTER SESSION

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	Poster Title	Student
PROCESSING	**Using cheese concentrates: understanding real cheese systems**	Agathe Lauzin, ULaval
	Using cheese concentrates: levers for controlling their performance	Iris Dussault Chouinard, ULaval
	Using cheese concentrates: approaches to facilitate the draining of curds	Isabelle Fournier, ULaval
	Biofilms in membrane systems: understanding and controlling their formation	Julien Chamberland, ULaval
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	Impact on cheddar production of lactic bacteria from raw milk associated with the use of silage	Mérilie Gagnon, ULaval
	The bioconservation of dairy products: think natural, think bioprotective bacteria	Hebatoallah Hassam, ULaval
	Reuterin: a natural antifungal with a high potential to replace Natamycin in the dairy sector	Helène Pilote Fortin, ULaval
	Enhanced exopolysaccharide production by *Lactobacillus rhamnosus* strains in coculture with *Saccharomyces cerevisiae*	Annalisse Bertsch, ULaval
	Improving the Greek yogurt production through an eco-efficiency approach based on the value chain	Catherine Houssard, Polytechnique
	New techniques to describe gel structure and serum entrapment in yogurt matrix	Audrey Gilbert, ULaval
	The structure and rheological properties of stirred yogurts are modulated by whey protein content and smoothing temperature	Audrey Gilbert, ULaval
	Pilot-scale test for smoothing stirred yogurts at different temperatures	Valérie Guénard-Lampron, ULaval
	Cheddar cheese ripening by metabolomic analysis	Pier-Luc Plante, ULaval
	Study of the typicity of Quebec local cheeses through metabarcoding	Annick Raymond-Fleury, ULaval
	Genomics characterization of isolates from the secondary microflora of Quebec cheeses	Gabrielle Jacquemet, ULaval
PRODUCTION	**Tall fescue: an interesting alternative to timothy grass in dairy cow rations**	Anne-Marie-Richard, ULaval
	Should we replace timothy grass in our alfalfa blends?	Florence Pomerleau, UMcGill
	Changing fodder grass: modelling of the impacts on the farm	Jean-Philippe Laroche, ULaval
	Effect of incomplete milking at the beginning of lactation to prevent infectious diseases and improve reproductive performance	Catarina Krug, UMontréal
	Improving health and fertility traits in dairy cows through an alternative genomic approach	Alexandra Carrier, ULaval
	Effect of tie-rail positions following the neck line of cows on the welfare of tie-stall housed cows	Jessica St John, UMcGill
	Increasing chain length for our cows in tie-stalls: a good idea?	Véronique Boyer, UMcGill

Using cheese concentrates: understanding real cheese systems

Agathe Lauzin[1], Iris Dussault-Chouinard[1], Michel Britten[2] and Yves Pouliot[1,*]

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Concentrating milk for cheese production is commonly achieved through ultrafiltration (UF). However, from an industrial eco-efficiency standpoint, other baromembrane processes such as reverse osmosis (RO) and nanofiltration (NF) are promising. The goal of this project is to characterize and compare the cheesemaking properties of UF, NF and RO concentrates to optimize their use in cheesemaking.

A number of cheesemaking properties of non-concentrated milk (SM) and UF, NF and RO (3X) concentrates were studied: the kinetics of coagulation through pressure and the impact of the pH of concentrates, their behaviour in a cheesemaking model as well as the kinetics of acidification through starter cultures, and the buffering capacity of concentrates.

The NF and RO concentrates presented significantly higher levels of minerals and lactose than UF and SM concentrates, resulting in a significantly higher viscosity and different coagulation properties: a significantly longer coagulation time than SM and UF, and a significantly lower firming speed than a UF concentrate. However, slight variations in pH (<0.2 pH unit) made it possible to re-establish the properties of NF and RO concentrates to obtain kinetics similar to that of a UF concentrate. The behaviour in a cheese model showed similarities between the three concentrates with regard to protein retention and adjusted yields but the humidity of NF and RO curds was significantly higher than for a UF concentrate. As the natural pH of the NF and RO concentrates is lower than for UF concentrates, the three concentrates needed the same amount of time to attain a target pH of approximately 5.2 despite the higher buffering capacities of NF and RO concentrates.

It therefore seems possible to obtain similar coagulation behaviours for the three concentrates through pressure and in a cheese model. It will nevertheless be necessary to deepen our understanding of these concentrates to better understand their behaviour in real cheese systems. Based on this knowledge, we will be able to consider their use in cheesemaking to improve the eco-efficiency of the cheesemaking food process.

Using cheese concentrates: levers for controlling their performance

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The use of reverse osmosis (RO) for the preconcentration of cheesemaking milk is an eco-efficient process since it generates a permeate with a low pollution load and reduces costs related to the transportation of milk. However, high lactose and mineral levels in RO concentrates reduces their cheesemaking abilities, requiring a correction of their physico-chemical properties prior to coagulation. To date, very few studies have focused on the levers for improving the performance of this dairy concentrate. The objective of this study was to evaluate the effect of two potential levers for improvement on the cheesemaking abilities of RO concentrates: the volumic concentration factor and acidification prior to renneting. Skim milk was concentrated through RO to four protein levels (5, 7, 9 and 11%), and then acidified at 4 pH levels (6.5, 6.2, 5.9 and 5.6) before receiving rennet For each protein level, a control concentrate, obtained through ultrafiltration, received rennet at a pH of 6.5. The amount of minerals and proteins in the concentrate and its soluble phase made it possible to determine the mineralization state of the casein micelles. The kinetics of coagulation through pressure were characterized through dynamic rheology and model cheeses were produced to study yield and composition. The results show that an increase in the protein level through RO slowed the coagulation mechanism, characterized by the increased ($p<0.05$) coagulation time and the plateauing of the gel firming speed at protein levels greater than 9%. An increased mineralization of the casein micelles and excessive humidity in the model cheeses were observed in comparison to the controls made of UF concentrates. The decrease in renneting pH proved to be a significant lever for correcting the RO concentrates by promoting a partial demineralization of the casein micelles, improved coagulation abilities, and better drainage. This new data will provide dairy processors with a tool for selecting the optimal pH/concentration combinations and facilitate the use of RO concentrates in cheesemaking.

Using cheese concentrates: approaches to facilitate the draining of curds

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Recent work has shown that the use of milk concentrated through reverse osmosis (RO) results in a significantly higher cheese yields when compared to skim milk (Lauzin et al., 2018). However, cheeses produced using this type of retentate present certain defects primarily related to their high water, lactose and mineral contents. The cheesemaking process could be adapted to the RO retentate to reduce humidity levels, as well as the mineral and lactose concentrations in cheeses. The goal of this project was to develop curd washing conditions that facilitate lactose removal, demineralization and draining.

The solution proposed to adapt the cheesemaking process to RO concentrates is to add a curd washing phase and to modify pH levels during racking. The curds are produced from a RO retentate with 7% of protein and with fat content (P/F=0.97) at four different pH levels (5.60; 5.80; 6.00; 6.20). The potential of washing curds with a reduced water content (15% $m_{water}/m_{retentate}$) was evaluated. Our first observations show that such modifications reduce the quantity of lactose present in cheese and facilitate curd draining.

Lastly, this project will provide viable solutions to industrialists who wish to use reverse osmosis retentates in cheesemaking. The results obtained on the pilot scale could be applied on the industrial scale, taking into account material constraints. Such a process will allow industrialists to increase their cheese yields and production efficiency. In addition, the production of a permeate with a zero-pollution load that could be reused in-plant constitutes a significant advantage from an environmental perspective.

Biofilms in membrane systems: understanding and controlling their formation

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Membrane filtration systems provide the perfect environment for the formation of biofilms. Biofilms are responsible for membrane fouling and dairy fluid contamination problems. In addition, they are difficult to eliminate from membrane systems due to an increase in their resistance over the course of cleaning cycles. As a result, this study aims to identify the ideal time to initiate the membrane cleaning cycle and to verify the impact of operating low-temperature systems to slow the development of biofilms.

A bioreactor-type system, continuously supplied with pasteurized skim milk, was used to stimulate the process of biofilm formation on the surface of ultrafiltration membranes. This system, equipped with eight 6-cm^2 membranes, was operated either at 50 °C for 20 h or at 15 °C for 48 h. The bacteria initially present in the milk circulating within the bioreactor, as well as the bacteria located on the membranes' surfaces, were identified through high through-put amplicon sequencing and quantified using qPCR at different time intervals.

It was observed that temperature significantly influenced the dynamics of the formation of biofilms on the membranes' surfaces. At 50 °C, the developing community was in an exponential growth phase after 10 h of operation, increasing from 1.47 to 6.71 $\log_{10}/cm^2$ after 12.5 h. In fact, the biofilm that was 99% dominated by the spore-forming bacterium *Bacillus* was in a stationary phase after 15 h of operation. At 15 °C, no bacterial genera were dominant in the communities and no exponential growth phases were observed during the 48 h of operation.

Our results therefore suggest that these two potentially applicable strategies could improve the economic and environmental performances of filtration systems by limiting the impact of biofilms. Reducing the operational temperature seems to be an efficient lever for slowing their short-term formation. Alternatively, to get the most benefit from the best filtration performance at 50 °C, it would be preferable to clean the membranes after less than 10 h of operation to target the less organized and less resistant communities.

Prevalence and diversity of microbial communities in silage and raw milk

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In the province of Quebec, more than 50% of dairy farmers use grass or corn silage to feed their cows. However, the effects of fermented fodder on the microbiological quality of raw milk are not well known. To determine the impact of silage on the microbiological structure of raw milk, 24 dairy farms in Quebec implementing different production typologies associated with inoculated grass and corn silages (GICI), grass and non-inoculated corn silages (GCNI), non-inoculated grass and non-inoculated corn silages (GNICI), non-inoculated grass silages (GNI) and hay (Hay) were recruited. According to the typologies, the hay, GI, GNI, CI and CNI samples (non-inoculated corn silage), as well as raw milk samples, were taken in spring 2015, fall 2015 and spring 2016. Their fungal communities were analyzed using high-throughput sequencing of the ITS2 region of the RNA gene. The number of species identified in the silages and the diversity levels, generally higher in non-inoculated silages (GNI and CNI), varied significantly ($p<0.001$) depending on the sampling period. A total of seven phyla were identified, with *Ascomycota* and *Basidiomycota* being most prevalent and abundant. Regardless of the type of silage, inoculation prevents the growth of certain mycetes while it seems to promote others. In addition to the species' genetics, the anaerobiosis rates in the silos or the acidity of the silages may explain this phenomenon. Raw milk with the GNI and GICI typologies present more diversified and abundant fungal communities. The distribution of species significantly varied according to the typologies and the sampling period. A taxon belonging to *Cordycipitaceae* significantly dominates the microbiota of raw milk with a proportion of 69-87%. In the different typologies, raw milk shares 46-56% of the unique species with silages or hay. The highest transfer rates for mycetes from silage to milk were observed in the GNICI and GICI typologies. The information drawn from this work will allow dairy farmers and processors to suggest management practices that may reduce the frequency of problems linked to milk contamination.

Impact on Cheddar production of lactic acid bacteria from raw milk associated with the use of silage

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Non-starter lactic acid bacteria (NSLAB) are commonly found in cheese. They play a major role in flavor development. However, heterofermentative LAB have been identified as a source of defects in cheese. In a previous study, we suspected that LAB present in raw milk may originate from silage. Silage is forage fermented by LAB with a high prevalence of heterofermentative LAB. Some strains were able to achieve passage from feed to milk. Our hypothesis is that milk NSLAB from farms using silage may be thermoresistant and therefore survive heat treatment to impact cheese making. Two strains, *Lactobacillus casei/paracasei* RKG R10 isolated after pasteurization of milk and *Lactobacillus plantarum* RKG 2-212 isolated from raw milk, were selected based on their higher levels of thermoresistance (heat shock at 60 °C for 5 min) compared to *Lactococcus lactis* subsp. *cremoris* SK11, *L. plantarum* ATCC 14917, and *Lactobacillus paracasei* ATCC 334 (F value: 15.9013; p-value < 0.0001). The two NSLAB strains were monitored in two Cheddar cheese models (manufacture: Pearce activity test; ripening: cheese slurry) in the presence of the reference starter *L. lactis* SK11. The two strains did not inhibit nor improve the growth of the starter during manufacture. In the cheese slurries, counts of *L. casei/paracasei* RKG R10 stayed stable during the 12 days at 30°C, while *L. plantarum* RKG 2-212 grew from 6.51 to 8.27 log of CFU/g of cheese slurry and acidified the slurry from 5.58 to 5.27. The volatile organic compound profiles of the slurries (SK11 alone; SK11+R10; SK11+2-212) were different. The presence of RKG 2-212 can be detrimental because of its production of acetic acid and 2-methylbutanal. In conclusion, the two strains isolated from raw milk taken from farms using silage are potential NSLAB contaminants because they are thermoresistant and exert an influence on Cheddar ripening.

The bioconservation of dairy products: think natural, think bioprotective bacteria

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The aim of this work is to study the interactions between bacteriocin-producing protective culture and starter culture used for cheddar cheese production. Three nisin A producing *Lactococcus lactis* subsp *lactis*, 5 nisin A resistant *Lactococcus lactis* subsp *cremoris* strains and 12 cheddar commercial starter were used in this study. Cell free culture supernatants (CFCS) from each bacterium was prepared under simulated cheddar cheese manufacture conditions prepared using skim milk and then used as a growth medium for the biocompatibility assay. The maximal growth rates of the *Lactococcus* strains (400 combinations) were analyzed by automated spectrophotometry. The final pHs and proteolytic activities were also examined at the beginning and at the end of simulated cheddar cheese processing. The results indicate that, four and five strains have high proteolytic activities and lower pH, respectively, compared to the control. On the other hand, all 8 Lactococcus strains showed normal growth curves in all CFWs. While five commercial starter have antagonistic interaction when inoculates into CFWs of the other strains including commercial starters. Based on these results, a consortium of three strains, two commercial starters and one nisin-producing strain, were selected to be used as starter protective culture during cheddar cheese production.

Reuterin: a natural antifungal with a high potential to replace Natamycin in the dairy sector

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The general objective of this work is to produce, purify and evaluate the antifungal activity of reuterin against moulds that are problematic for the dairy sector. Reuterin was produced by fermenting glycerol in anaerobic conditions through *L. reuteri* ATCC53608. Purification was completed through HPLC and the inhibitory activity of the supernatant and pure reuterin was evaluated through agar diffusion against the sensitive strain *Listeria innocua*ATCC25922. Reuterin's antifungal activity in milk was evaluated on four alteration fungal strains (*Aspergillus niger*, *Mucor racemosus*, *Penicillium chrysogenum* and *Rhodotorula mucillaginosa*) through agar diffusion and in an RPMI medium. Lastly, the antifungal activity was evaluated in stirred yogurt samples conserved at 4 °C for 28 days in a DRBC medium. The purified reuterin demonstrated significant antifungal activity on agar at concentrations of 125 mM for *A. niger*, *R. mucilaginosa* and *P. chrysogemum*, and 15 mM for *M. racemosus*. The MIC in an RPMI medium is 3.83 mM for *p. chrysogenum* and *R. mucillaginosa* and 1.91 mM for *A. niger*. The calculation of the FMC/MIC ratio revealed fungicidal activity. The results of the preliminary tests in stirred yogurt revealed a complete inhibition of the two moulds tests during the 21 days of conservation at concentrations of 6.9 mM and greater. These preliminary results demonstrate the antifungal potential of reuterin and its potential use as an alternative to chemical antifungals in yogurts.

Enhanced exopolysaccharide production by *Lactobacillus rhamnosus* strains in coculture with *Saccharomyces cerevisiae*

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Several *Lactobacillus* strains can produce exopolysaccharides (EPS) with recognised health benefits (i.e. prebiotic and immunomodulatory properties). The industrial EPS production by lactic acid bacteria (LAB) is limited by low yields. Co-culture appears as a new alternative to improve metabolite productivity, particularly bacteriocin or EPS (kefiran). Although LAB and yeasts are found in several fermented products, the molecular mechanisms linked to the related microbial interaction and their influences on EPS biosynthesis are unclear. The present study aimed to investigate the effect of co-culture on EPS production by three *Lactobacillus rhamnosus* strains (ATCC 9595, R0011, RW-9595M) in association with *Saccharomyces cerevisiae*. Fermentations, either mono or co-culture, were carried out using whey permeate in culture media without pH control and quantitative expression analysis of target genes of LAB was carried out by RT-qPCR during the process. Results revealed no significant differences in growth, sugar consumption and lactic acid production in co-cultures compared to mono-cultures ($p>0.05$). Interestingly, EPS production was enhanced by 39 and 29% in co-culture fermentations for ATCC 9595 and RW-9595M, respectively ($p<0.05$). For all the target genes, significant differences in their expression were found during co-culture compared to monoculture fermentations ($p<0.05$). Each strain showed distinctive gene expression profiles. For a higher EPS production, higher EPS operon expression levels were observed for RW-9595M in co-culture. Construction of gene co-expression networks revealed interactions between the EPS operon, metabolic and stress-related genes, for each strain ($p<0.01$). Our findings provide substantial insights into the mechanisms regulating EPS biosynthesis. The absence of pH control makes it possible to simplify the fermentation process while having an improvement in the production of EPS in co-culture which allows the reduction of input costs and at same time the biological recycling of whey permeate.

Improving Greek yogurt production through an eco-efficiency approach based on the value chain

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Greek yogurt (GY) has disrupted the yogurt market over the last decade. Its texture and high protein content are traditionally obtained by concentrating fermented milk through centrifugation. However, increasing volumes of acidic whey are difficult to valorize, engendering new economic and environmental costs for dairy processors. Recent work has been completed on new production approaches to reduce and/or eliminate these large volumes of acidic whey. Our project aims to evaluate these solutions from a sustainable development perspective by developing an innovative eco-efficiency (EE) tool. This model adopts a systemic vision based on the life cycle assessment (LCA) and takes into account the interests of different actors in the value chain (dairy farmers, processors, consumers) to answer the question: "What is the most eco-efficient way to valorize a ton of milk to produce GY in Quebec from a societal perspective?"

Three options for production (centrifugation (CE), protein fortification (PF) and ultrafiltration (UF)) and whey valorization (animal feed, biodiesel, food ingredient) to produce nonfat GY with 10% protein were retained following an industry consultation. The environmental impacts of these options were then calculated using a process simulation software from Université Laval and LCA software. In Quebec's current context, where nonfat milk solids (NMS) remain poorly valorized, our results based on a systemic approach suggest that the production of nonfat yogurt through CE or UF could have a positive environmental impact by allowing for the use of NMSs while producing the cream in demand on the market. In addition, the PF option (with imported MPC80) has more impact than other options, and optimizing the protein retention rate in GY during the concentration step reduces the impact of each option.

The results of this project will provide dairy processors with a concrete measuring tool and new perspectives to evaluate the impact of their production systems on Quebec society.

New techniques to describe gel structure and serum entrapment in yogurt matrix

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Investigating yogurt's water retention is a challenge. Low-frequency NMR (^{1}H-LF-NMR) is a non-destructive technique to potentially better understand syneresis phenomena.

This study aimed to understand the link between yogurt microstructure and syneresis using ^{1}H-LF-NMR in different dairy matrices.

Experimental dairy protein solutions, experimental yogurt from commercial pasteurized skim milk, and commercial stirred yogurts were analyzed with ^{1}H-LF-NMR. After a mathematical transformation of the signal, hydrogen atoms pools were differentiated according to their mobility. Each hydrogen pool stood for a type of water mobility in the matrices characterized by a relaxation time ($T_2(i)$), and a signal intensity ($I_2(i)$). Yogurt water retention was assessed by induced syneresis (centrifugation) and their structure was characterized using microscopy.

During Induced syneresis measurement, it is not possible to differentiate spontaneous syneresis from the serum that was expelled during centrifugation. Low frequency NMR detected four different water mobility groups in matrices. Among these, there was a signal from the serum, and another one only found in yogurts that came from separated serum (spontaneous syneresis). In yogurts, serum mobility is reduced with the increase of protein content or the increase with the protein network density. In commercial yogurt without gelatine, having a dense and homogeneous network, induced syneresis and serum mobility were low. In experimental yogurts, for which serum was separating spontaneously, induced syneresis was higher than 50 % agreeing with spontaneous syneresis detected by ^{1}H-LF-NMR. In presence of gelatine, the network was more open, which favored serum mobility. However ^{1}H-LF-NMR did not detect spontaneous syneresis anymore, which explained the low induced syneresis results.

This study showed that ^{1}H-LF-NMR associated with digital microscopy efficiently assesses and describes yogurts water retention and spontaneous syneresis.

The structure and rheological properties of stirred yogurts are modulated by whey protein content and smoothing temperature

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Consumers are not always ready to compromise with the loss of texture and increased syneresis that non-fat stirred yogurt displays.

In this study, milk protein composition and smoothing temperature were investigated as a leverage to control the non-fat yogurt microstructure, textural properties and wheying-off.

Protocol: Yogurts were prepared with different caseins to whey proteins ratios (Y1.5, Y2.8 and Y3.9). Yogurts were pumped through a smoothing pilot system comprising a plate heat exchanger set at 15, 20, or 25 °C and stored at 4°C until analysis (day 1, 9 and 23). Yogurt particle-size (microgels; analysis of microscopic images), and firmness (texture analyzer) were measured. Yogurt syneresis and water mobility were respectively measured by centrifugation and time domain low frequency ^{1}H-NMR (^{1}H-LF-NMR).

Increasing the smoothing temperature increased gel firmness and microgel sizes independently of the whey protein content. Also, yogurt microgel sizes evolved with storage time, but the evolution pattern depended on protein ratio. Yogurt Y1.5 showed the biggest particles and their sizes increased with storage time as opposed to Y2.8 and Y3.9. Micrographs showed heterogeneous gel with empty area occupied by serum for Y1.5, while Y2.8 and Y3.9 showed few serum and more disrupted gel embedding microgels. Forced syneresis reduced with increase whey protein content and time of storage. It is in agreement with 1H-LF-NMR showing less serum mobility with increasing whey protein content during storage. However, high spontaneous serum separation was observed during storage for Y1.5 and Y3.9 while it was low and stable for Y2.8.

Microgels play an important structural role on yogurt textural attributes and they are modulated by whey protein content and smoothing temperature. Optimization of these parameters by the dairy industry may limit the use of additives in non-fat dairy products improving consumer satisfaction.

Pilot-scale test for smoothing stirred yogurts at different temperatures

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Smoothing is an essential operation when stirring yogurt. It provides a smooth product, but causes significant breakdown of the protein network. The effect of smoothing temperature on the rheological properties of yogurt has been poorly studied. The goal of the project was to determine and model the impact of 6 smoothing temperatures using a pilot-scale unit simulating industrial conditions on a nonfat stirred yogurt.

Yogurts were stirred under the same conditions but cooled to different temperatures (10, 15, 20, 25, 30 and 35 °C) using a plate heat exchanger and were then smoothed and cooled to 10 °C using a tubular heat exchanger. Syneresis, apparent viscosity, firmness, flow resistance and flow time were analyzed during 22 days of storage at 4°C. The behavior of yogurts was described by multiple linear regressions and a sensitivity analysis allowed quantifying the impact of temperature and storage on the studied properties.

Multiple linear regressions adequately described the behavior of syneresis, firmness, and flow time ($0.81 \leq R2 \leq 0.94$). Syneresis increased with smoothing temperature (from 10 °C to 35 °C), while the other properties also tended to increase, but only up to 30 °C. Unlike firmness and flow time, syneresis, viscosity and flow resistance were more sensitive to temperature variation than to storage variation.

This study contributed to a better understanding of the impact of the smoothing temperature and demonstrated that there is no specific temperature that can optimize all yogurt properties.

Cheddar cheese ripening by metabolomic analysis

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The process of Cheddar cheese ripening is already well documented and mastered by numerous cheesemakers. Unfortunately, the cheese maturation process is not always as good as expected, particularly at the organoleptic level. Indeed, sometimes the flavour of the final product is not up to the expected standards of quality. We speculate that the metabolomic analysis of a fresh cheddar cheese could help predicting the fate of aged-cheddar cheeses through the early identification of specific metabolites. We also hope to identify novel metabolic makers that are associated with a high-quality aged cheddar.

We used an untargeted metabolomic approach in order to find metabolites for which the abundance increase or diminish with the cheese ripening process. We developed a sample preparation method to extract metabolites and lipids from cheddar cheese samples that is compatible with liquid-chromatography coupled with a mass spectrometer instrument (LC-MS).

When using the developed method, the analysis of different cheeses at different maturation time allowed us to find metabolites linked to the ripening process. Also, the addition of organoleptic properties information to the statistical analysis allowed to find metabolites correlated to some typical cheese flavours.

The validation process of the metabolites is ongoing and will probably require more cheese samples. We believe that our approach will allow to confirm the impact of some metabolites in the ripening process of cheddar cheese. In the end, we hope to provide a list of metabolites that can be monitored in real time during the maturation of the final product. The results of this project could help our dairy industry to better control the quality of their product and reduce the losses linked to decommissioning. Furthermore, it could be possible to reduce the storage cost by predicting the quality of the final product in order to exclude some cheeses from the long-aging process.

Study of the typicity of Quebec local cheeses through metabarcoding

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The cheesemaking industry represents a large part of Quebec's economy. In the province of Quebec, annual production exceeds 230,000 tons of cheese, with 28% made up by fine cheeses. Between 2% and 20% of these cheeses are affected by quality problems, including a lack of consistency caused by an imbalance in their microflora. While the microflora plays a major role in the development of a cheese's sensory properties, very little information is available on its composition (presence and relative abundance) for cheeses from Quebec. The goal of this study is to develop a molecular biologic method to create a complete characterization (bacteria and mycetes). After preliminary studies were completed to determine the best DNA region to use, the bacterial characterization (region V6-V8 of rDNA 16S) as well as the fungal characterization (region ITS2 (internally transcribed spacer–ITS)) of 50 cheeses were completed. At the cheese's core, the bacterial microflora (*Lactobacillales* order - *Lactococcus* and *Lactobacillus* genera) and fungal microflora (*Saccharomycetales* order - *Geotrichum*, *Debaryomyces* and *Kluyveromyces* genera) consist mainly of starter and ripening cultures. On the surface, the microflora is typical of the type of cheese studied (bloomy, mixed or washed rind). For example, the bacterial microflora of washed rind cheeses is composed of psychrohalotolerant microorganisms (*Alteromonodales*, *Oceanospirillales* and *Pseudomonodales* orders - *Pseudoalteromonas*, *Cobetia* and *Halomonas*, *Psychrobacter* genera), while the fungal microflora of bloomy rind cheeses is dominated by typical ripening cultures (*Eurotiales* and *Saccharomycetales* orders - *Penicillium* and *Geotrichum* genera). The analysis method developed also allowed us to evaluate the variations in the microflora of 17 cheeses from two different production years (2015 and 2018). The persistence or lack thereof of dominant microorganisms was evaluated and strategies can now be developed to better control the cheeses' microflora. Lastly, this new knowledge will enable the production of high quality cheeses in a consistent manner and make Quebec more competitive in relation to foreign cheeses, all while reducing economic losses related to the downgrading of fine cheeses presenting sensorial defects.

Genomic characterization of isolates from the secondary microflora of Quebec cheeses

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To stay competitive in an increasingly open market, Quebec cheesemakers must optimize the quality and unique taste of their fine cheeses. The characterization of the secondary microflora of numerous cheese varieties has previously revealed the presence of microorganisms (bacteria, yeasts, moulds) from the secondary microflora which could contribute to the development of sensory properties (taste, odour, texture) of fine cheeses. Understanding the role of these non-inoculated microorganisms during the ripening process is therefore essential. A characterization of the complete genome of strains from Quebec will make it possible to identify the species' genes and specific metabolic pathways, and to establish their individual roles in the production of aromatic compounds. Based on the complete genome sequence, the main objective of this study was to better understand the metabolic potential of 12 bacteria and 6 yeasts isolated from the secondary microflora of cheeses or cheesemaking milk from Quebec. To do so, microorganisms that were previously identified through metagenomics were isolated from cheeses and cheesemaking milk using selective environments. Strains belonging to the *Halomonas*, *Marinomonas*, *Cobetia*, *Psychrobacter*, *Staphylococcus*, *Glutamicibacter*, *Debaryomyces*, *Cyberlindnera*, *Pichia*, *Kazachtania* and *Rhodotorula* genera were sequenced on Illumina MiSeq and HiSeq platforms. For one of these genomes, *Staphylococcus equorum*, the analysis was completed for the purposes of gene prediction (using the Prodigal software program) and functional annotation (by using the COG and KEGG databases). This bioinformatic method will then be used to characterize all of the isolates to gain the most ample knowledge of their technological capacities. This new knowledge will increase our understanding of the role of certain species in the secondary microflora—for example, their ability to produce aromatic and flavour precursors—in addition to providing cheesemakers with tools to optimally promote or control their development during the cheese ripening stage.

Tall fescue: an interesting alternative to timothy grass in dairy cow rations

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Timothy grass is the most popular feed grass in Quebec, but dry conditions and higher temperatures in summer limit its regrowth. Tall fescue seems to be an interesting alternative since it can better tolerate these summer conditions. Nevertheless, some farmers fear its use due to its unpopularity. The objective of this project was to evaluate the effect of replacing timothy grass with tall fescue, provided as a pure species or mixed with alfalfa. The project also aimed to verify the impact of the conservation method used to preserve tall fescue, either as haylage (35% dry matter, DM) or semi-dry silage (55% DM), on the performance of lactating cows.

The experiment was completed with 15 Holstein cows in a lactation environment. Five treatments were tested: 1) timothy grass (70% of the ration, DM base) in haylage; 2) timothy grass (42% of the ration) + alfalfa (28% of the ration) in haylage; 3) tall fescue (70% of the ration) in haylage; 4) tall fescue (42% of the ration) + alfalfa (28% of the ration) in haylage; 5) tall fescue (70% of the ration) in semi-dry silage.

Food intake, milk production and the milk's fat content were the same for cows fed with timothy grass or tall fescue-based rations. When the two feed grasses were offered mixed with alfalfa rather than as a pure species, food intake and milk production were higher, while the milk's fat content was lower and its protein content was similar. Despite a decreased food intake when tall fescue was served in the form of semi-dry silage rather than as haylage, milk production remained the same. Our results confirm the possibility of using tall fescue as a replacement for timothy grass in dairy cow rations and that it is advantageous to offer the feeds mixed with alfalfa rather than as a pure species.

Should we replace timothy grass in our alfalfa blends?

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Timothy grass is the grass that is most commonly associated with alfalfa in Quebec, even though it has limited regrowth in hot and dry conditions. In the near future, these hot and dry conditions are expected to worsen due to climate change. To find the best alternatives to timothy grass in our current climatic conditions, six binary associations of grasses and alfalfa were evaluated at three sites in Quebec (Sainte-Anne-de-Bellevue, Saint-Augustin-de-Desmaures, Normandin). The alternative grasses evaluated were: tall fescue (cv. Carnival), meadow fescue (common seed), perennial rye grass (cv. Remington), festulolium (cv. Spring Green), and meadow bromegrass (cv. Fleet). The parcels were harvested at two stages of the alfalfa's development—at the beginning of budding and the start of flowering—and the yield, nutritional values, and contributions to the yield of the different species seeded were measured.

The alfalfa-timothy grass, alfalfa-meadow fescue, and alfalfa-meadow bromegrass mixes were correlated with the highest yields, nutritional values, and estimated milk production per hectare of fodder crop; the meadow fescue and meadow bromegrass are therefore suitable alternatives to timothy grass when mixed with alfalfa. Despite its lower nutritional value, the alfalfa-tall fescue mix is also a probable alternative since it had a seasonal yield and estimated milk production per hectare similar to the alfalfa-timothy grass mix. Timothy grass, meadow fescue, tall fescue and meadow bromegrass proved to be productive during the first three years of production. However, the mixes studied consisting of alfalfa with the festulolium and perennial rye grass cultivars had lower yields and estimated milk production per hectare, in addition to contributing less to the yields; these cultivars are therefore not reasonable alternatives to timothy grass in Quebec. With regard to harvesting practices, the cutting completed at the beginning stages of flowering, compared to the cutting completed at the beginning of the alfalfa's budding stage, increased yields and the estimated milk production for the alfalfa-grass binary mixes.

Changing fodder grass: modelling of the impacts on the farm

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The climatic warming affecting Quebec's agricultural regions and the expected variations in precipitation will affect fodder crop production on dairy farms. Some regions of Quebec are already beginning to see the repercussions of these changes. Timothy grass, the grass most commonly used in fodder mixes in Quebec, may be affected since its regrowth is weak in the summer's hot and dry conditions. Other fodder grasses should therefore be considered as alternatives to timothy grass. Using modelling, the objective of this project was to evaluate the impact of the choice of alfalfa-based binary associations in rotations on the profitability of farms. The N-CyCLES farm optimization model was used. It was adapted to account for the average technico-economic data from two dairy farms, one of which was located in the Lower St. Lawrence region and the other located in Montérégie. Four fodder associations were tested: alfalfa + timothy grass; alfalfa + tall fescue; alfalfa + meadow fescue; and alfalfa + meadow bromegrass. The yields and nutritional values of the binary associations for each of the regions were adapted based on the data from a test completed at three sites over three years of production (Pomerleau-Lacasse et al 2018), as well as a Valacta database.

Cutting management practices related to the alfalfa's persistence and balance between the nutritional value and yield were considered. For the Lower St. Lawrence farm, the alfalfa + tall fescue association performed best with a net benefit of $13.00/100 kg of milk corrected to 3.8% fat and 3.3% protein (FPCM) compared to $12.20/100 kg of FPCM for alfalfa + timothy grass, $11.90/100 kg of FPCM for alfalfa + meadow fescue, and $11.60/100 kg of FPCM for alfalfa + meadow bromegrass. The variations in net benefit were lower in Montérégie with a maximum variation of $0.40/100 kg of FPCM calculated between the associations. In the current climatic conditions, our analysis of the farms therefore indicates that the alfalfa + tall fescue association is a potential alternative for the alfalfa + timothy grass association. This new knowledge concerning the performance of different fodder grasses associated with alfalfa will allow dairy farmers in Quebec to make more enlightened decisions. Extrapolating these results for future climatic conditions, through modelling, will make it possible to establish recommendations for optimizing the sustainability of Quebec dairy farms.

Effect of incomplete milking at the beginning of lactation to prevent infectious diseases and improve reproductive performance

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Incomplete milking during the first five days of lactation (DOL) reduces the risk of hyperketonemia and therefore helps to prevent the risk of infectious diseases and improve the reproductive performance of multiparous dairy cows. The objective of this study was to evaluate the effect of this practice on health.

Multiparous cows (n=853) from 13 commercial farms were enrolled in a randomized controlled trial and were randomly distributed in a control group (conventional milking) or incomplete milking group (maximum 10-14 L of milk/day) between DOL 1 to 5. Milk samples were collected to identify intra-mammary infections (IMI) and cases of clinical mastitis were recorded. The cows were examined for the presence of uterine diseases (DOL 35). Blood samples were taken to determine progesterone concentrations (DOL 33 and 47) and the resumption of luteal activity. Reproductive data was also extracted from electronic health records. The effect of incomplete milking with regard to rates for new IMIs, the elimination of existing IMIs, uterine diseases, and the resumption of luteal activity, as well as on the time until clinical mastitis and the time between the end of the voluntary waiting period (VWP) and successful breeding, was modelled.

Regarding infectious diseases, the IMI elimination rates of cows following an incomplete milking protocol were higher than for cows subjected to conventional milking (2.9 times higher, CI 95%: 1.4 to 6.0). Regarding reproductive performance, cows subjected to an incomplete milking appeared to perform better than cows subjected to conventional milking. In effect, cows following an incomplete milking protocol had a probability of conception 205.8 (CI 95: 109.1 to 388.0) times higher during the 21 days following the end of the VWP. This probability was 16.4 (CI 95%: 9.9 to 27.3), 4.7 (CI 95%: 2.8 to 8.2), 1.9 (CI 95%: 1.1 to 3.4), and 0.23 (CI 95%: 0.15 to 0.35) times that of cows subjected to conventional milking, respectively, between days 22-43, 44-65, 66-87, and >87 following the end of the VWP.

These results suggest that an incomplete milking during DOL 1 to 5 promotes the elimination of IMIs and has a positive effect on cows' reproductive performance.

Improving health and fertility traits in dairy cows through an alternative genomic approach

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Dairy farmers seek functional animals with a long productive life, whereas health and fertility traits demonstrate weak heritability and slow genetic progress. It is known that an impoverished genetic diversity can have negative impacts on an animal's health and reproductive abilities. To improve these traits, diversifying the genome may be more beneficial than the homogenization that is currently taking place. The approach used in this project consists of diversifying the genome and, therefore, only a small percentage of the genome is targeted. A group of approximately 200 bulls were genotyped and the regions associated with health and fertility traits were identified using information existing in the scientific literature. The regions were then selected using an algorithm correlating the animals' performance with the genotypic information. The health and fertility score was also tested out of a population of 1,000 cows wherein the individuals demonstrating distinct phenotypes designated as being sick or healthy were used. Based on the list of markers identified, we can establish a genetic diversity score for each animal. With the analysis completed on the bulls, high correlation values (0.33 to 0.51) were found for most of the health and fertility traits. The results demonstrate that dairy production traits are not affected. The analysis completed on the cows' health data demonstrated a significant statistical trend between the score and the animals' state of health. In addition, a mating management tool was developed to provide dairy farmers with a list of bulls that demonstrate better genetic complementarity for a given cow according the cow's genotype. This tool will be economically beneficial in that it will reduce culling rates caused by health and fertility problems, as well as costs for veterinary services to treat diseases. It will also result in increased well-being for the animals.

Effect of tie-rail positions following the neck line of cows on the welfare of tie-stall housed cows

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The majority of dairy farms in Canada house their cows in tie-stall barns, however few experimental studies have investigated ways to improve cow comfort through tie-stall design. Studies on Canadian dairy farms have found that tie-rail position has an affect on lying behaviour and neck injuries. Cows often come into contact with the tie-rail when they are rising and eating. The objective of this study was to develop new recommendations for tie-rail placement to reduce these contacts, by positioning the tie-rail to follow the neck line of cows when they are rising and eating. Three tie-rail positions were compared to current recommendation (height: 48 in.; forward position: 14 in.): two new positions (44 in.; 7 in. and 40 in.; 14 in.) and the tie-rail position most commonly found on farm (48 in.; 7 in.). Forty-eight cows, grouped by parity and days in milk, were randomly allocated to a treatment for 10 weeks. Live injury scoring was performed 1 d/wk. Cows were recorded 1 d/wk by cameras to evaluate lying and rising ability.

Our results indicate an increase in neck injuries higher on the cows' neck when the tie-rail is positioned higher, lower on the cows' neck when the tie-rail is positioned lower, and both higher and lower on the cows' neck when the tie-rail is positioned at an intermediate height. The tie-rail position did not affect the rising and lying ability of the cows, however rising and lying ability did improve over time, suggesting that the cows adapted to their housing conditions. Future research on metal bar alternatives such as chains or a more flexible bar should be investigated further. Such alternatives may improve dairy cow welfare long term if they help reduce neck injuries and provide solutions to help farmers fulfill the requirements of the proAction® program.

Increasing chain length for our cows in tie-stalls: a good idea?

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While tie-stalls are the predominant system used on Canadian and Quebec dairy farms, very few studies have examined the issue of improving cow comfort in this type of housing, let alone in an experimental context. By responding to the question "Do cows benefit from a longer chain than the recommended length?", this study aims to fill the gap in the literature and validate the current recommendation for chain length.

To do so, 24 cows from McGill University's Macdonald campus farm were divided into groups according to their number and stage of lactation and assigned for a period of 10 weeks to one of two treatments: the current recommendation (1.00 m, control group) and a longer chain (1.40 m). The other aspects of the stalls' configuration corresponded to the current recommendations in the Code of Practice.

Each cow was filmed one day/week using video cameras positioned above the stalls, and six events (standing up or laying down) per day were evaluated. Injuries to the neck, knees and hocks were recorded each week to monitor their evolution over time. Milk production was also evaluated based on the data automatically collected during each milking, and milk samples were taken each week.

The results indicate that neither injuries nor production were affected by either of the treatments. The lay-down preparation phase was shorter for cows with a longer chain, which indicates that they are more comfortable in their environment.

As a result, although certain measures targeted in the proAction® program, such as injuries, do not seem to be affected by chain length, at least during the 10-week study, the fact remains that this modification could be a simple and cost-efficient way to provide dairy cows with a space in which they are more comfortable, thereby increasing opportunities for movement provided by the stall and improving their level of well-being.

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