

TECHNO - LOGICAL FORUM 2016

Novalait

Research Catalyst

A word from the President

On behalf of the Board of Novalait, it is a great pleasure to welcome you to this special edition of Novalait Technological Forum.

Novalait is a unique business model. Novalait exists because dairy farmers and dairy processors of Quebec share a vision. In Quebec, all dairy farms and all companies that transform milk, from the artisan cheesemaker to the multinational, support Novalait. Together, they annually contribute to research up to 1.27¢/hl of milk traded. They mandated Novalait to invest these funds for developing the knowledge, technology and expertise needed to produce and process the milk of the future.

Last year, Novalait gathered you to mark 20 years of research. Today, 105 projects compose our research portfolio representing \$ 10 million invested by Novalait on a total value of research of 45.4 million. Thanks to its private and government partners, Novalait generates a quadruple leverage of the investment of its shareholders. This unique business model enables dairy companies to share risks and costs of research they could not afford individually.

With this exceptional record, Novalait continues to innovate particularly in communication. A new website facilitates access to our research results. Novalait also took a bilingual turn. Now videos, capsules and all Novalait communication tools will be developed in French and English. I would like to take this opportunity to especially welcome our shareholders and Anglophone colleagues attending the Forum for the first time. We wish to start a new tradition of exchanges on the results and research opportunities. We believe that discussions between researchers, dairy farmers and processors are essential to identify applications and potential benefits of research results, particularly in terms of cost reduction and of economic, environmental and social benefits.

The training of highly qualified professionals is one of the most concrete benefits from research. At least a third of the budgets of Novalait's researches is reserved for student wages. The Forum provides a unique opportunity to appreciate their expertise and discover their career aspirations. The excellence of their performance at the poster session will be highlighted by the awards for students.

In closing, I invite you to enjoy all the opportunities of the Forum to discuss your research interests, on the most recent results from projects and perhaps recruit new expertise to innovate!

To you all, I wish you a fruitful Forum!

Réal Gauthier,
President of Novalait

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Program

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8 h 00 Registration

8 h 40 Welcoming remark

Dairy Processing – Room 3
Ms Élise Gosselin, Novalait CEO



Dairy Production - Room 2
Mr Réal Gauthier, Novalait's President



8 h 45 Research results and benefits – Rooms 2 and 3

Moderator : Ms Carole Thibault, Danone

8 h 45 **How to enrich dairy products with polyphenols and increase health benefits**
Mr Laurent Bazinet, Laval University

9 h 10 **Better understanding the role of the milk typicity in the quality of fine cheeses**
Mr Steve Labrie, Laval University

9 h 35 **Do mechanical treatments have an impact on the technological quality of milk?**
Mr Michel Britten, AAC-CRDA

10 h 00 3 minutes thesis competition

The bacterial profile of biofilms formed on filtration membranes : the influence of an increasing selective pressure within the dairy plant, Julien Chamberland

Environmental analysis of whey valorisation
Angela Maria Trivino

Simulation of industrial production of stirred yogurt at pilot scale, Valérie Guénard-Lampron

Moderator : Ms Geneviève Rainville, PLQ

Assessing the lifetime profitability of a cow combining production and health data
Mr Roger Cue, McGill University

B vitamins, the rumen bacteria, are they always sufficient to the task?
Ms Christiane Girard, AAC-CRDBLP

How the mineral profile of the diet influences the milk fat?
Ms Édith Charbonneau, Laval University

3 minutes thesis competition

The role of minerals in the production chain of milk fat, Angel Rene Alfonso-Avila

Control of negative energy balance and consequences of incomplete milking in early lactation, Catarina Krug

The milk fatty acid are talking to us,
Eric Baumann

10 h 20	Health break - Poster session - Room 1
11 h 10	Vision and leadership in dairy research - Rooms 2 and 3
11 h 10	Developing together solutions and expertise to produce and process the milk of the future Ms Élise Gosselin, Novalait CEO
11 h 30	<u>New research initiative on comfort, longevity and sustainable life of dairy cows</u> Ms Elsa Vasseur, McGill University
12 h 00	Lunch - Room 1
13 h 00	Poster session - Room 1
13 h 45	Conferences – Consolidate expertise – Rooms 2 and 3 Moderator : Mr Simon Robert, Agropur cooperative
13 h 45	Keynote speaker : Milk Production and Processing Innovation : Meeting Public Expectations Mr Dave Barbano, Professor, Food Science and Director, Northeast Dairy Foods Research Center, Cornell University
14 h 30	<u>Industrial research chair on nutritional control of the production of milk components in cows</u> • Review and benefits Mr Yvan Chouinard, Laval University
15 h 00	<u>Industrial research chair in efficiency of milk processing</u> • Measuring and improving eco-efficiency in dairy processing : from laboratory to the plant Mr Yves Pouliot, Laval University
15 h 50	Excellence awards
16 h 00	Closing Remark Mr Réal Gauthier, Novalait's President
16 h 15	Wine and cheeses

Factsheets

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How to enrich dairy products with polyphenols and increase health benefits

Duration : 2011 – 2016

Highlights

- This project is part of an initiative to identify the nutritional synergies and protective effects of dairy products on nutrients in other food groups.
- In regards to objective 1, this study is the first of its kind to demonstrate that the presence of calcium has a large influence on the interaction between EGCG/ protein β Ig and EGCG/ protein alpha s1 casein. In the case of beta-Ig, the presence of EGCG is required in order to have an increased particle size in accordance with increased calcium concentrations.
- In objective 2, the study demonstrated that milk has the strongest fixation ability for polyphenols from green tea with a rate that is slightly higher than 80%, while the average fixation rate of yogurt is 75% for polyphenols. However, cheese matrices have a greater protective effect on polyphenols during digestion.
- According to these results, calcium supplementation is important for milk that is enriched with strong concentrations of extracts rich in polyphenols. In addition, it appears that polyphenols influence the digestion of dairy products by decreasing the rates of liberation of peptides and fatty acids.
- The results confirm for the very first time that dairy matrices have a protective effect on the antioxidant activity of polyphenolic compounds during digestion. However, polyphenols in cranberries are more sensitive to the gastro-intestinal environment than polyphenols in green tea.
- Concerning objective 3, the results support that β -Ig has beneficial effects on glycemic control but suggest that the consumption of EGCG and calcium do not increase this effect.

*EGCG: epigallocatechin-3-gallate

Objectives

In order to study the nutritional synergy between milk components and polyphenols contained in tea and cranberry juice, this study focuses on three main objectives:

- To characterize the interactions between milk components and isolated phenolic compounds in tea and cranberry juice;
- To measure, during digestion, the effect of milk components and three dairy matrices (milk, yogurt, cheese) on degradation kinetics and antioxidant activity of phenolic compounds;
- To measure, post digestion, the effect of the presence of milk components on the physiological activity of polyphenols.

Results and potential benefits

Research on these three components has primarily helped to advance knowledge in the dairy sector since many of the results have been demonstrated for the very first time. In addition, the knowledge and results obtained from this project will contribute to the development of new polyphenol-enriched dairy products while demonstrating the beneficial effect of the addition of polyphenols on health or during digestion. With the knowledge acquired during this project on the interactions between polyphenols/calcium/dairy proteins (alpha 1 casein and β Ig), new dairy ingredients may be developed (protein aggregates rich in EGCG and calcium). Depending on the interests of dairy processors, these results may be used as part of a knowledge transfer project conducted in-plant. The results concerning the effect of polyphenols from grapes (commercial extracts) on rennet-induced coagulation could also be validated in-plant beforehand. In addition, results demonstrate that it is possible to preserve the antioxidant activity of

polyphenols according to the type of dairy matrix and the manner in which they are incorporated. Lastly, consumers benefit from knowing this information about the liberation of polyphenols during the digestion of milk matrices, as well as about the protection and liberation of polyphenols, as the research demonstrates the real impacts and benefits of milk components (beneficial effect on postprandial glycemia). The potential benefits of the research results are: 1) Increased revenues due to an increased demand for dairy products: Reinforcement of the “healthy” image of milk. In addition to its high nutritional density, milk provides “protection” to nutrients from other food groups (nutritional synergies); 2) Increased product quality: Effect of milk proteins and calcium on the protection and liberation of polyphenols during gastrointestinal digestion; and 3) The ability to better respond to consumers’ concerns.

Contact persons

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Professionals trained

Martine Lussier: Master's degree (incomplete)

Expertise acquired:

- Methods of analyzing polyphenol-protein interactions
- Methods of analyzing antioxidant properties (DPPH, ORAC)
- Fractioning techniques and characterization of proteins found in dairy products
- Dosage techniques
- Operation of a static digestion simulator

Valérie Carnovale: PhD (completed)

Expertise acquired:

- Methods of analyzing polyphenol/calcium/milk protein interactions
- Effects of dairy products and their interactions on physiological responses
- Physical chemistry of proteins
- Physiology and digestion of dairy products

For further information

• Lussier, M.; Britten, M.; Couillard, C.; Bazinet, L. Interaction entre les polyphénols d'un extrait de thé vert et les matrices laitières: impact sur les activités anti-radicalaire et anti-oxydante. Poster presented during the first research day at the Faculty of Agriculture and Agri-Food Sciences, "Mieux nourrir le monde." Quebec City (Qc), November 28, 2012.

• Lussier, M.; Britten, M.; Couillard, C.; Bazinet, L. Interaction entre les polyphénols d'un extrait de thé vert et les différentes forme de matrices laitières. Poster presented during the STELA Symposium "Innovation for the Future of the Dairy Sector" and the FIL-IDF "Dairy Outlook Seminar," Montreal (Qc), May 13–16, 2013.

• Carnovale, V.; Britten, M.; Couillard, C.; Bazinet, L. Étude et impacts des interactions entre l'épigallocatechine gallate, la bêta-lactoglobuline et le calcium dans la phase minérale du lait. Poster presented during the Novalait Technological Forum "Carrefour des compétences, savoirs et savoir-faire laitiers," Drummondville (Qc), May 28, 2014.

• Lussier, M.; Britten, M.; Couillard, C.; Bazinet, L. Interaction entre les polyphénols d'un extrait de thé vert et les matrices laitières. Poster presented during the Novalait Technological Forum "Carrefour des compétences, savoirs et savoir-faire laitiers," Drummondville (Qc), May 28, 2014.

• Carnovale, V.; Britten, M.; Couillard, C.; Bazinet, L. Effet de complexes épigallocatechine-3-gallate et la β -Lactoglobuline formé dans un ultrafiltrat de lait simulé avec et sans calcium sur la réponse métabolique chez la souris. Poster presented during the 2015 STELA Symposium "Challenges and Opportunities for the Dairy Sector." Poster No. 27. Quebec City (Qc), June 1–2, 2015.

• Carnovale, V.; Labaey, C.; Britten, M.; Couillard, C.; Bazinet, L. Impact du calcium sur les interactions entre épigallocatechine-3-gallate et la β -Lactoglobuline. Poster presented during the 2015 STELA Symposium "Challenges and Opportunities for the Dairy Sector." Poster No. 28. Quebec City (Qc), June 1–2, 2015.

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: \$250 000

Better understanding the role of the milk typicity in the quality of fine cheeses

Duration : 2012 – 2016

Highlights

In Quebec, locally produced products are becoming increasingly important to consumers. This project is one of the rare undertakings exploring the characteristics of locally produced milk and the characterization of indigenous fungal species in fine cheeses.

The project has allowed us to:

- Establish that indigenous yeasts from local products have unique characteristics that allow them to survive and either compete or work in synergy with starter cultures;
- Identify the flavour compounds generated by these microorganisms, creating the possibility of inoculating cheeses with the species studied in order to control flavour production;
- Demonstrate that there is a local influence since some milk from the same breed of cow differed in composition and fermentability depending on the origin of the milk;
- Demonstrate that a starter culture composed of multiple lactococcal strains may behave differently depending on whether it is used in raw or pasteurized milk; and
- Generate new information allowing us to keep records of cheesemakers

Objectives

The general objective of the project is to increase microbiological and technological knowledge about local milks in order to improve and regulate the quality of fine artisanal cheeses from Quebec and identify their unique characteristics.

Obj. 1. Verify the influence of the indigenous fungal microflora of milk on the cheese ageing process.

Obj. 2. Determine the impact of secondary fungal microflora on the flavour profiles of cheeses.

Obj. 3. Determine the components of milk that influence the growth of fungal microflora involved in the ageing process and secondary microflora.

Results and potential benefits

Major findings. The interactions between 12 indigenous yeast strains of raw milk and two Fungi used in the cheese ageing process were characterized. A molecular quantification method (gPCR) was optimized for some of the strains to evaluate the distribution of species in local cheeses. These species were also analyzed to examine their contribution to cheese flavour profiles. Floral, malted and rancid flavours were also associated with some of the indigenous yeast species. We also compared the composition of milk from different breeds (Canadian, Jersey, Brown Swiss, Holstein and industrial) and different regions of Quebec, as well as their impact on the growth of lactococcal strains and yeasts. For a single breed, the origin of the milk (region/locality) had a non-negligible influence on composition. In general, the lactococcal

strains grew better in milk from Brown Swiss cattle. Pasteurization of the milk improves growth rates (1 additional log) and lactic acid production is 4 times higher than in raw milk. It was demonstrated that some lactococcal strains only required the presence of caseins to grow while others also required the presence of serum proteins.

Economic and social impacts. This study allows for a better understanding of the impact of milk composition and indigenous microflora on the properties of local cheeses. This information helps us to identify the particularities of milk produced locally and the key indigenous species that have an impact on cheese quality.

Contact persons

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Professionals trained

Joanie Côté, M.Sc. candidate. Technological and physico-chemical properties of different types of milk (microbiology, physical chemistry)

Andréanne Lamarche, M.Sc. candidate. Distribution of local yeast strains (microbiology, molecular biology)

Ariane Pelletier, M.Sc. candidate. Optimization of an identification technique for flavours produced by local yeast strains (microbiology, analytics)

Annick Raymond-Fleury, B.Sc. student. Optimization of the barcoding method for rapid identification of milk yeasts and moulds (microbiology, molecular biology)

For further information

- Scientific articles concerning the detection of local yeast strains and the production of volatile compounds from the strains (in the process of being written)
- Presentations in the form of posters at the 2016 IDF Cheese Science & Technology Symposium

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

Andréanne Lamarche and Ariane Pelletier have received scholarships from the Canadian Dairy Commission in collaboration with Novalait. Andréanne Lamarche has received a scholarship Agro:Inno from INITIA, CTAQ and INAF.

Total budget: \$220 000

Do mechanical treatments have an impact on the technological quality of milk?

Duration : 2012 – 2017

Highlights

This project aims to study the impact of mechanical treatments on the technological quality of milk intended for cheese production. The effects of light treatments (pumpage, skimming) and intense treatments (churning, high pressure homogenization) are also studied.

- Pumpage and skimming lead to a low level of flocculation of the milk fat globules and the absorption of a small quantity of surface proteins;
- The characteristics of casein micelles, coagulation properties and cheese yields are not influenced by milk pumpage and skimming conditions;
- High pressure homogenization of skimmed milk leads to the solubilization of colloidal calcium, reduces the size of casein micelles and increases their electric charge;
- Despite its effects on milk properties, high pressure homogenization was shown to have no impact on its coagulation ability and cheese yields;
- Churning leads to major modifications in the light cream phase (buttermilk) which significantly decreases its technological qualities;

The research that is currently underway aims to study the potential of partial homogenization of cheesemaking milk to modulate cheese characteristics.

Objectives

The general objective of the project is to demonstrate the effect of mechanical treatments on the physico-chemical properties of milk and its cheesemaking ability.

The specific objectives are to measure the effect of 1) pumpage; 2) skimming; 3) churning; 4) high pressure homogenization of skimmed milk; and 5) partial homogenization of whole milk.

Results and potential benefits

Cheesemakers are aware of the importance of optimizing the technological qualities of milk intended for cheese production. In a highly competitive environment, improving yields and controlling cheese composition is essential. Standardizing milk and mastering heat treatments have been the subject of many studies. In comparison, the impact of mechanical treatments on milk's cheesemaking ability has not received much attention.

Our work will make it possible to quantify the effect of mechanical treatments on the dispersion state of fats, the physico-chemical properties of casein micelles and the balance between the colloidal and soluble phases. Despite the changes observed and the preciseness of

analysis methods, no significant impacts on the coagulation ability of milk or cheese yields have been recorded. The only exception concerns the churning of cream, which produces buttermilk whose fractions' technological qualities are inferior to those of milk.

The next portion of the project aims to evaluate the partial homogenization of cheesemaking milk as a control tool for cheese composition and characteristics. The presence of a fraction of homogenized fat in milk will modify the structural organization of the curd, its syneresis and the cheese's properties (composition, texture, melting properties).

Contact persons

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Michel Britten

Agriculture and AgriFood Canada

Professionals trained

Marie-Pierre Gauvin (PhD)

Expertise acquired:

- Production and fractioning of buttermilk;
- Dosage techniques;
- Analysis techniques (electrophoresis, high-performance liquid chromatography (HPLC), inductively coupled plasma (ICP), granulometry, rheology);
- Coagulation kinetics; characteristics of rennet gels; cheese production in a model system.

Marie-Pier B. Vigneux (M.Sc.)

Expertise acquired:

- High pressure homogenization;
- Dosage techniques;
- Analysis techniques (electrophoresis, high-performance liquid chromatography (HPLC), inductively coupled plasma (ICP), granulometry, rheology);
- Coagulation kinetics; characteristics of rennet gels; cheese production in a model system.

For further information

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

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Financial contribution

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: \$ 204 000



Assessing the lifetime profitability of a cow combining production and health data

Duration: 2011 – 2014

Highlights

- Cow longevity is a known problem which results in too many expensive herd replacements, and a sub-optimal proportion of mature cows in the herd. This results in lower productivity and, consequently, profitability. Producers have separate access to milk-recording information (Valacta) and to veterinary health event data (e.g., DSA); however, they lack an integration which could provide a lifetime view of a cow's profitability and her herd mates (i.e., benchmarks).
- This project combined production (Valacta) and health event (DSA) data to model and demonstrate the value of an integrated cumulative lifetime profit perspective, and to examine the factors affecting culling rates and longevity.
- A prototype visualisation tool was developed to show the lifetime profit of a cow or group of cows in a herd compared to a suitable contemporary average. The prototype tool also allows for a comparison of a herd versus a benchmark average of comparable herds.
- Profitability (calculated to the end of the fourth lactation) can be reduced by as much as 20% due to health events (e.g., mastitis, ketosis, displaced abomasum, feet and leg problems, repeat breedings and long dry periods); hence these are important factors to consider when deciding which cows to keep and which to cull.
- The visualisation tool also demonstrates – in a very visual manner – the impact of delayed age at first calving on lifetime profitability, as well as the time required to pay off the rearing costs of a heifer.
- The prototype software and model consider revenues and costs at the individual cow level and can, therefore, use accumulated information to provide a profile for the herd.

Objectives

General objective: To combine Valacta and DSA data to produce lifetime profit records for each cow and hence demonstrate the impact of health events on overall individual cow profitability and longevity.

Hypotheses:

- a failure to integrate production and health-event information results in under-estimating the overall impact of health events;
- the integration of production and health event information, on a lifetime basis, can provide useful comparative and benchmark statistics, and allow a producer to better evaluate the profitability of the cows in his/her herd;
- the presentation of the results in a visual and graphical form can aid in understanding what particular factors or time points in a cow's life are impacting overall productivity, and hence should be focussed on in an effort to improve profitability.

Results and potential benefits

Results obtained :

1. Development of a methodology and software program to combine production information from Valacta (e.g., milk production, feed information and breeding information) with DSA health event information (e.g., mastitis, reproductive problems, feet and leg problems etc.);
2. Development of a profitability model based on production and health events; and
3. Development of a prototype software to visualise the lifetime profit of a cow and a benchmark comparison, indicating the various events (e.g., calvings, breedings, health events, dry periods, etc.).

The results of this research and development project can aid dairy producers and their advisors to reduce veterinary-health costs by showing them the overall impact of each cow in a particular herd, thus motivating the producer to prevent problems. It is expected that sensitising producers to the cost and impact of health events will allow them to reduce

milk loses and costs associated with somatic cell count penalties (or at least enable producers to benefit from the premiums for low cell counts). Being able to show the overall impact of events during the lifetime of a cow can assist a producer in working to improve productivity as well as to improve overall animal welfare and health. Having a tool to show the impact of health events, specific to a particular farm, can help the producer respond to concerns and preoccupations of the general public concerning animal welfare and wellbeing. These results should allow the dairy industry to see the increased benefits of combining all available data (e.g. production, veterinary health events, hoof trimming and cow comfort, etc.) to provide better profiles of cows and herds, thus improving on-farm management. The results of this research can be applied in the short to medium term, and are primarily applicable for an on-farm management tool for producers and their advisors.

Contact persons

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Professionals trained

Two doctoral students (**Hector Delgado** and **Denis Haines**) were trained as part of this project. They acquired expertise and training in the analyses of large data sets, epidemiological methods, interpretation of field-level data, and presentation of scientific results had scientific conferences.

For further information

Several scientific presentations were given at conferences (e.g., American Dairy Science Association, Bovine Veterinary Practitioners) as well as poster presentations at the Symposium sur les bovins laitiers and at the forum Technologique of Novalait. In addition to a poster presentation at the Symposium sur les bovins laitiers (2015) the prototype software visualisation tool was also available for demonstration purposes. Scientific articles from both doctoral students are in preparation for high-quality journals, and an extension article has appeared in the Producteur de Lait (in addition to an informative short video on the Novalait web site).

Financial contributions

Collaboration agreement for innovation in dairy production and dairy processing (ECI2008-2014):

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

Total budget: \$ 246 897



B Vitamins: are rumen bacteria always sufficient to the task?

Duration : 2011 –2016

Highlights

- The Vitamin B needs of dairy cattle are not always met through the synthesis of vitamins by bacteria in the rumen.
- In these conditions, vitamin B supplements increase lactation performance and the metabolic efficiency of dairy cattle.
- The results of this project show that concentrations of B vitamins in food do not predict the amounts available to the cow and that these amounts vary widely depending on the composition of the ration.
- A database has been compiled and is ready to be used to develop a model that can be used to predict the amounts of B vitamins available to the cow depending on the nutritional management strategy.
- This model will allow producers to modify their nutritional management strategy according to the cow's needs and, if necessary, to evaluate whether or not vitamin B supplements should be used.
- Over the long term, taking this data into account using formulation software for dairy cattle should result in increased metabolic efficiency, thereby reducing production costs and environmental waste. This will lead to increased efficiency within agricultural enterprises in Quebec and reduce their environmental footprint.

Objectives

- Hypothesis: It is possible to predict the amount of B vitamins available to cows according to the chemical composition of the ration.
- Objective: To determine the dietary supply of B vitamins, synthesis in the rumen and the flow of vitamins to the duodenum in different nutritional conditions. In addition, compile the data for the future development of a predictive model for vitamin B supply in dairy cows that can be integrated into ration formulation software to maximize metabolic efficiency in dairy cattle.

Results and potential benefits

A number of studies have shown that the vitamin B needs of dairy cows are not always met. In these conditions, vitamin B supplements increase lactation performance and the metabolic efficiency of dairy cattle. However, there is very little information that allows us to predict the conditions in which vitamin B supply is negatively or positively affected. This project has demonstrated that it is possible to predict the amount of B vitamins available to cows according to nature of the diet. The project has shed light on the factors influencing the availability of B vitamins to dairy cows. In conjunction with the studies allowing us to quantify cows' needs for B vitamins, this data will help us to identify the conditions in which dietary supplements may be necessary to meet the animal's needs.

The information collected and the database that was compiled as part of this project, once integrated into a predication model, will allow us to specify the vitamin(s) to be added to the ration as well as the dose to be used. Taking this data into account using formulation software for dairy cattle should result in increased metabolic efficiency, thereby reducing production costs and environmental waste. This will lead to increased efficiency within agricultural enterprises in Quebec and reduce their environmental footprint.

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Valérie Beaudet, master's student in animal sciences; analysis of B vitamins in complex biological environments, research assistant at the Agriculture and Agri-Food Canada centre in Sherbrooke.

Douglas de Souza Castagnino, PhD student in animal sciences, analysis of B vitamins in complex biological environments, database construction, postdoctoral fellow in modelling at the University of Guelph.

For further information

- Castagnino, D.S., K.L. Kammes, J.A. Voelker Linton, M.S. Allen, R. Gervais, P.Y. Chouinard, M. Seck and C.L. Girard. 2015. La synthèse apparente de thiamine et de vitamine B12 dans le rumen de vaches laitières recevant une ration à base d'ensilages de luzerne ou de dactyle. In: Science information day—Dairy cattle and forage plants. Centre de référence en agriculture et agroalimentaire du Québec. Drummondville, Qc, 25 February. http://www.craaq.qc.ca/documents/files/Documents/EBOV1502/castagnino_oral.pdf
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Financial contributions

Collaboration agreement for innovation in dairy production and dairy processing (ECI2008-2014):

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

Total budget : \$250 000



How the mineral profile of the diet influences the milk fat content?

Duration : 2012 – 2016

Highlights

Research research has shown that the synthesis of fat content in milk fat from cows at the beginning of lactation receiving a ration rich in concentrates is affected by the mineral profile of the ration, particularly stimulated by the addition of a source of potassium carbonate (K_2CO_3).

This research project therefore aims to:

- Determine if the effects observed on production performance are due to an increase in:
 - K inputs;
 - the dietary cation-anion difference (DACA); or
 - buffering capacity;
- Evaluate the effects of the addition of K_2CO_3 to acidogenic rations (rich in concentrates and vegetable oil).

The results of this project have allowed us to observe that:

- There is a wide variability between animals in regards to the ability of their rumen to resist rations rich in concentrates and to maintain a high fat content;
- The addition of K_2CO_3 to the ration has an effect on the bacteria involved in the biohydrogenation of fatty acids in the rumen;
- An increase in K concentrations in the ration through the addition of K_2CO_3 leads to an imbalance in mineral ions, causing a decrease in production.

Objectives

- Separate out the effects of the DACA, K content and buffering capacity of the ration on the biohydrogenation of polyunsaturated fatty acids in the rumen and milk fat content.
- In high-producing cows receiving a ration rich in concentrates, verify the interaction between K_2CO_3 and soybean oil (source of polyunsaturated fatty acids) inputs on:
 - the rumen's microbial populations;
 - the biohydrogenation of fatty acids; and
 - animal performance.

Results and potential benefits

The results of this study allowed us to verify there is a wide variability between cows from the same herd in regards to the ability of their rumen to resist rations rich in concentrates. In addition, we observed that the addition of K_2CO_3 to the ration leads to modifications in bacterial populations, which then prevent rumen synthesis of fatty acids and inhibitors of the milk fat synthesis. However, contrary to the results obtained by other research teams, the current experiment does not allow us to associate an increase in the DACA and/or potassium concentrations from the ration with an increase in the daily production of milk fats. Lastly, this study allowed us to observe that the increase in K content through the addition of K_2CO_3 to the ration causes an imbalance that negatively affects animal production, specifically milk production.

Advances in scientific knowledge regarding the impact of the mineral profile of the ration on animal performance helps us to better identify feeding strategies that have a real impact on milk production and composition in high-producing dairy cattle. The data collected also gives us a better understanding of the importance of the ionic balance of the ration on the mammary gland's ability to produce milk. These results will allow us to develop innovative nutritional strategies that, once integrated into feeding systems, will have significant economic benefits for producers.

Contact persons

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Valacta

Alain Fournier

Ministère de l'Agriculture, des Pêcheries
et de l'Alimentation du Québec

Professionals trained

Angel Rene Alfonso Avila, a PhD candidate, has developed unique expertise in the study of microbial populations and rumen metabolism, and assessing the use of minerals for dairy cattle.

For further information

Alfonso-Avila, A.R., É. Charbonneau, P.Y. Chouinard, G.F. Tremblay, and R. Gervais. 2015. Utilisation du carbonate de potassium pour maximiser la production de matières grasses du lait. Page 18. In: 39th Dairy Cattle Symposium. Centre de Référence en Agriculture et Agroalimentaire du Québec, October 29, 2015, Drummondville, Qc.

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praticiens du Québec, September 24-27, Saint-Sauveur, Qc.

Alfonso-Avila, A.R., P.Y. Chouinard, E. Charbonneau, J. Chiquette, G.F. Tremblay, A.-M. Richard and R. Gervais. 2016. Nutrition minérale: Nouvelles perspectives pour des performances laitières optimales. Forum Technologique Novalait. June 2. Hôtel et Suites Le Dauphin, Drummondville, QC.

Charbonneau, E., A.R. Alfonso-Avila, P.Y. Chouinard, J. Chiquette, G.F. Tremblay, A.-M. Richard and R. Gervais. 2016. Influence du profil minéral de la ration sur la production de matière grasse du lait. Forum Technologique Novalait. June 2. Hôtel et Suites Le Dauphin, Drummondville, QC.

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

Centre de recherche en sciences animales de Deschambault

Total budget : \$220 000

Novalait

Research catalyst



New research initiative on comfort, longevity and sustainable life of dairy cows

Duration : 2016 – 2020

Highlights

- Dairy farmers have the challenge of providing the market with high quality products while ensuring maximum productivity. To do so, understanding the nutritional and physiological needs of cows, while simultaneously taking consumer expectations into account in regards to the environment, animal welfare and economic accessibility, is crucial.
- Increasing the longevity of dairy cattle in order to decrease involuntary cullings and reduce economic losses for farmers and the industry, all while respecting concerns about animal well-being and comfort and the environment, is a major issue.
- For these reasons, and to develop expertise in this field, the new research chair in the sustainable life of dairy cattle was created at McGill University. This important research initiative aims to provide dairy farmers with concrete data and tools to optimize comfort, with a particular focus on stall housing, and to increase the longevity of dairy cattle.
- The chair also aims to improve recommendations concerning animal welfare to help farmers prepare for the implementation of the animal welfare component of the ProAction national program.

Objectives

- The research chair's objective is to optimize comfort, with a particular focus on stall housing, and to increase the longevity of dairy cattle while considering dairy farm sustainability.

Results and potential benefits

The chair will conduct its research activities with a focus on three main themes over the next five years.

Theme 1: Cow comfort and herd management

- The first research theme will concentrate on the knowledge and adaptations required to provide the appropriate environment for dairy cattle with respect to compliance. As current research on comfort is essentially conducted with loose housing systems, projects will be completed using a tie-stall system in order to better understand comfort needs and optimize recommendations.

Theme 2: Dairy cattle longevity

- The second research theme will examine long-term profitability measures and animal survival in herds. The topics of replacement and producing

cows will be examined. The projects completed under this theme will benefit from the data collected on commercial farms as well as from Valacta's database.

Theme 3: Environment and society

- The third research theme will help provide a broader perspective on international standards, life cycle assessments and consumer acceptability. Measures to improve welfare and longevity in accordance with overall sustainability (environmental, economic and social) of dairy farms will be validated.

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Novalait
Research catalyst

Professionals trained

The research chair has just been appointed (January 1, 2016) and a PhD student (**Elise Shepley**), three master's students (**Jessica St John**, **Sirine EL Hamdaoui**, **Véronique Boyer**) (all focusing on welfare and comfort) and a postdoctoral fellow (**Hector Delgado**; large database analysis) have already begun or are about to begin their research programs at McGill University. In total, the chair's research projects will involve more than a dozen graduate students and more than twenty interns.

For further information

- A first introductory article about the chair will appear in the June 2016 edition of Le producteur de lait québécois
- Website for the chair:
<https://www.mcgill.ca/animal/staff/elsa-vasseur>

Financial contributions

The research chair is funded under the program of the National Sciences and Engineering Research Council of Canada (NSERC) Industrial Research Chair;

Industrial Partners are:

- Novalait
- Dairy Farmers of Canada
- Valacta

McGill university also contributed financially.

Total budget: \$ 1 720 000

Industrial research chair NSERC-Novalait- DFC-PLQ- MAPAQ-Valacta on nutritional control of the production of milk components in dairy cows

Duration : 2009 – 2016

Highlights

- This research program was established to help dairy farmers better manage the marketing of their products. It aims to help farmers meet consumers' needs and develop control mechanisms for milk production and composition that align with the objectives set by the dairy industry.

As a result of this chair's research:

- It is now possible to assess the impact of the ration on rumen balance based on the milk's fatty acid profile.
- The impacts of different nutritional strategies on milk synthesis and composition (dietary cation-anion difference (DACA), the ration's fatty acid profile, lipid supplementation) were validated in commercial conditions.
- Tools for improving the transfer of ω -3 fatty acids from the ration to milk and methods for preventing the oxidation of fatty acids that are beneficial to health were identified.
- Advanced techniques now allow us to characterize volatile compounds that determine milk's flavour profile.

Objectives

- Improve management and feeding techniques for dairy herds in order to better control the production of milk components.
- Modify the proportions of different nutritional elements in milk in response to market needs.
- Evaluate the effects of a cow's diet on milk's organoleptic qualities.

Results and potential benefits

By providing producers with additional tools to better predict the nutritional value of rations and/or quickly diagnose certain metabolic disorders by analyzing milk's fatty acid profile, the results of this research have direct impacts on herds' diets and, consequently, the enterprise's profitability. In addition, by improving the transfer of ω -3 fatty acids from the ration to milk and developing ways to prevent their oxidation, it is possible to improve the input of these essential fatty acids, not only in dairy products for consumers, but also in cow tissue. The research has also made it possible to validate, in commercial conditions, the concepts developed as a result of observations made in experimental conditions, such as, for example, the importance of the dietary cation-anion difference (DACA), the addition of lipid

supplements, and the fatty acid profile of the ration on milk production and composition. The results obtained are directly applicable to farms and will allow Canadian producers to more effectively manage the fat content of the milk produced by their company and thereby increase their revenues.

By contributing to the development of new techniques to determine milk's flavour profile, this research allows for better control over the volatile compounds in milk. This will allow for the development of niche products with unique flavour profiles. In the long-term, this knowledge can also be used to develop a diagnostic service for producers whose milk is rejected by truck drivers due to bad odours.

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Professionals trained

Rachel Gervais, Ph.D., post-doctoral fellow: development of diagnostic tools based on the analysis of the fatty acid content of milk. Professor in the Department of Animal Sciences at Université Laval.

Adriana Pilar Urviola Garcia, graduate student intern—Chili: Modulation of milk composition in relation to market needs.

Marie-Christine Fauteux, M.Sc.: transfer of carotenoids from dried fodder in milk secretions and the impact on oxidative stability of fat content in milk. Animal product consultant, La Coop.

Maxime Leduc, M.Sc., Ph.D. candidate: modulation of minor components in milk in relation to market needs.

Hernan José Bueno Larroque, graduate student intern—Uruguay: Modulation of milk composition in relation to market needs.

Sara Maritza Pena Cotrino, graduate student intern—Uruguay: Modulation of milk composition in relation to market needs.

Hanen Manai, M.Sc., Ph.D. candidate: effects of dietary fatty acids on fat content in milk in commercial conditions.

Jair Esteban Paraes Giron, graduate student intern—Columbia: Modulation of milk composition in relation to market needs.

Liliana Fadul Pacheco, Ph.D. candidate: Study of dietary factors influencing major components of milk in commercial conditions through database analysis.

Eric Baumann, M.Sc., Ph.D. candidate: development of diagnostic tools based on the analysis of the fatty acid content of milk.

Marie-Pier Villeneuve, M.Sc.: effects of fodder type on the flavour profile of milk. Post-secondary teaching in agriculture.

Leacady Saliba, M.Sc.: Effects of polyunsaturated fatty acids interacting with starch in rations on the lactone content of milk and the impact of flavour compounds on milk flavour. Pursuing doctoral studies abroad.

Daniel Rico, Ph.D., post-doctoral fellow: Flavour and control of the oxidative stability of milk. Plans to pursue a career in dairy production research in Canada.

For further information

23 presentations to partners

- *Novalait Technological Forum, 2015*
Dairy Cattle Symposium, Science information day—Dairy cattle and forage plants, STELA Colloquium

18 presentations at scientific conferences

- *American Dairy Science Association, International Symposium on the Nutrition of Herbivores*

7 scientific publications

- *Journal of Dairy Science, Animal, Journal of Dairy Research*

4 popular science articles

- *Le producteur de lait québécois, The Milk Producer*

Novalait
Research catalyst

Financial contributions

National Sciences and Engineering Research Council of Canada

Dairy farmers of Canada

Les Producteurs de lait du Québec

Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec

Novalait

Valacta

Centre de recherche en sciences animales de Deschambault

Université Laval

Total budget : \$1 631 086

Industrial research chair NSERC-Novalait in efficiency of milk processing: Measuring and improving eco-efficiency in dairy processing: from laboratory to the plant

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 producers 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 **implementation 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.

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Novalait
Research catalyst

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

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) 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

Factsheets for ongoing projects

Factsheets for ongoing projects

Poster Session	Scientist
 Adjusted milking during the transitional period to better control negative energy balances and its consequences	Simon Dufour, Université de Montréal
 Development of an innovative green biotechnology for the valorization of cheese co-products : The Biobac process	Michèle Heitz, Université de Sherbrooke
 Prevalence of microorganisms in silage and raw milk and their impacts on dairy product quality	Denis Roy, Université Laval
 Impact of the dynamics of the process and composition of fermented dairy products on their stability and rheological qualities	Sylvie Turgeon, Université Laval
 Improving fodder grass in the context of climate change	Edith Charbonneau, Université Laval
 Impact of production processes on the microbiologic quality of Greek yogurt and the valorization of bi products	Gisèle Lapointe, Université Laval
 Systems biology applied to cheddar production	Sylvain Moineau, Université Laval
 Improving eco-efficiency in milk processing by optimizing the usage of milk components: the case of Greek yogurt	Yves Pouliot, Université Laval

Adjusted milking during the transitional period to better control negative energy balances and its consequences

Duration : 2013 – 2016

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 level of ketone bodies in the blood between the 6th and 15th days of lactation.
- Cows subject to incomplete milking seem to adopt a desirable resting behaviour (i.e., time lying down) earlier in the lactation period.
- The impact of incomplete milking on the incidence of infectious and metabolic diseases and on reproduction will be studied in the coming months.

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 will help us confirm the usefulness of an innovative energy balance management method during the transitional period in a commercial context. Our hypothesis is that adjusted milking will temporarily reduce milk production and energy needs in multiparous cows in the transitional period and thereby effectively manage the negative energy balance and acetonemia regularly observed in these animals. In addition, these improvements will not have a negative impact on the cows' subsequent milk production. We believe that this increased control of the energy balance will help reduce the incidence of many health problems, improve reproductive performance and, ultimately, lead

to an increased longevity of dairy herds. The anticipated improved control of health problems and reproductive performance may even result in an increase in subsequent milk production.

In addition, we expect that this method will have a positive effect on the profitability of herds, given the minimal cost and major positive impacts potentially associated with this alternative management method during the transitional period. Lastly, the results of this research on multiparous cows will potentially help direct future research to evaluate the impact of the management method on the performance of primiparous cows.

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Novalait
Research catalyst

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 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 results of this research will be communicated in the near future (i.e., upon completion of the research) to dairy producers. We will be focusing on articles in

Les Producteurs de Lait Québécois, Le Savoir Laitier and The Milk Producer and giving trainings and presentations for users together with our partners at Valacta as part of the Op+Lait FRQ-NT (www.oplait.org) research group's knowledge transfer activities.

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

Development of an innovative green biotechnology for the valorization of cheese co-products: the Biobac process

Duration : 2013 – 2016

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 bacteria.
- 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 bacteria *Escherichia coli*. Mutations inactivating the key genes of five competing fermentation pathways were generated, thereby increasing the yields of both products and decreasing the production of sub-products.
 - The initial results of the fermentation of whey and whey permeate on a laboratory scale produce diol yields of 19% and 12%, respectively (solute mass/solution mass). We are currently optimizing the fermentation conditions.
 - In order to verify the economic and environmental feasibility of the valorization of whey and its permeate, a life cycle assessment in combination with an energy efficiency index assessment is underway.
 - Three sizes of cheese companies were assessed. Five co-product valorization scenarios were considered, including the valorization of whey protein concentrates (WPC), biogas and the new Biobac process evaluating the valorization of whey and its permeate. These comparisons will allow us to identify the most efficient processes from an economic and environmental perspective.

Objectives

General objective: Genetically modify a bacteria 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), the diol yield is 21% (m/m) after 20 hours of fermentation, while it is 18% (m/m) after 24 hours with a whey

concentration of 12.5% (g/L). In identical operating conditions, the diol yield obtained from whey and its permeate is less than 20% (m/m).

In regards to the assessment of environmental impacts and energy efficiency, the points addressed concern: a) the valorization of dairy co-products that may have commercial value, providing a sustainable supply source as an alternative to petroleum; b) an improved quality of life and the protection of natural resources (water) by reducing the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of industrial waste produced in dairy processing; c) the development of references for life cycle analysis in order to calculate the energy efficiency of the valorization of whey and its permeate. These points will constitute the first study to compare a valorization process for cheesemaking co-products in Quebec; and d) the study of the life cycle assessment and energy efficiency assessment in order to identify the processes or critical steps and create strategies that reduce the environmental impacts.

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CNETE

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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 review of the literature on the production of butanediol through fermentation has been submitted (Fernandez et al.)
- International conference: Fernandez et al. 2016. Fermentation of a dairy waste into acetoin by *Escherichia coli*. 8th International Conference on Waste Management and the Environment, Wessex, June 2016, Valencia, Spain
- An article describing the development of strains of *Escherichia coli* capable of producing acetoin and butanediol is currently in preparation (Rousseau et al.)
- An article describing the operating conditions for the fermentation of glucose and lactose in the presence of a modified strain is in preparation (Fernandez et al.)

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

Novalait
Research catalyst

Prevalence of microorganisms in silage and raw milk and their impacts on dairy product quality

Duration : 2013 – 2017

Highlights

- Milk is a major staple for human consumption.
- 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 5 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 producers identify the best silage management practices in order to optimize the microbiological quality of milk and help processors control the sources of microbial contaminants.

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**
 - Better identify and quantify the microorganisms that are present and active.
 - Better understand the transfer rate of bacteria and mycetes from silage to milk.
 - Better understand the role of lactic acid bacteria inoculants on the processing of milk into cheese.
- **Effects of milk's indigenous flora on the organoleptic properties of processed dairy products**
 - Better understand the relationship between the organoleptic characteristics of processed milk and the heat-resistant bacteria of interest or bacteria causing alterations.

- Predict the specific metabolic characteristics (thermo-resistance and the production of bacteriocins) through the comparative analysis of the genome of isolated lactic acid bacteria.

Potential Benefits

- **Economic**
 - Optimization of the microbiological quality of milk used in cheesemaking
 - Control of the organoleptic quality of dairy products
- **Environmental**
 - Reduction in numbers of rejections of poor-quality processed dairy products
- **Social**
 - Maintaining quality standards for milk and dairy products

Contact persons

Project managers :

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Robert Berthiaume

Valacta

Thibault Varin

(Postdoctoral student)
Université Laval

Professionals trained

Mérlie Gagnon (doctorat)

Phenotypic and genotypic characterization of isolated lactic acid bacteria in silage and raw milk: antibacterial activity, thermoresistance and the production of volatile compounds. Mérlie Gagnon has acquired expertise in: i) isolating and identifying lactic acid bacteria in different types of fodder (hay, non-inoculated silage and inoculated silage) and in raw milk from cows feeding on the fodder; 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 (doctorat)

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

A results characterisation chart will be discussed with the Novalait steering committee in order to identify if some discoveries should receive protection in collaboration with the Vice-Rector for Research and Creation at Université Laval. The results of the project will be distributed through presentations and posters at the general assembly of the Op+LAIT strategic group, The Forum Techno and at national and international conferences. The conclusive results will also be published in scientific journals with the review committee. They will also be used to write general science articles intended for the industrial sector.

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

Novalait
Research catalyst

Impact of the dynamics of the process and composition of fermented dairy products on their stability and rheological qualities

Duration : 2013 –2016

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.
- Expected results: Identify the critical points in the yogurt production process; understand the composition characteristics that are important for shearing resistance and the ability to re-form a high 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

Composition characteristics: shearing resistance and the ability to re-form a high 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.

Identify the critical points of the yogurt production process

- 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.

Improving 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.

Contact persons

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Professionals trained

Currently, three master's students are being trained in this project.

- **Valérie Guénard-Lampron** (MSc#1: obj. 1.2) is completing a thesis on the effect of the conditioning parameters of stirred yogurt on its rheological properties. Expertise: physical chemistry, processes
- **Noémie Lussier** (MSc#2: obj. 2.2) is completing a thesis on the 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) is completing a thesis on the impact of cooling speed on the stability and rheological properties of stirred yogurt. Expertise: physical chemistry, processes

Currently, one doctoral student is being trained in this project.

- **Audrey Gilbert** (not funded by the project) (PhD#1: obj. 2.1) is completing a thesis on the impact of process dynamics and the composition of fermented dairy products on their functional properties. Expertise: physical chemistry

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 presented:

- Guénard-Lampron, V., S. Grabowski, G. Bélanger, L.P. Desmarchais, S. Villeneuve, D. St-Gelais, S.L. Turgeon. 2015 Conception et mise en oeuvre d'un banc d'essai à l'échelle pilote pour étudier l'effet des opérations de production sur les propriétés du yogourt brassé. STELA Colloquium, Quebec City, Canada, June 1–2
- Gilbert, A., L.E. Rioux, D. St-Gelais, S.L. Turgeon 2015 Effet des cisaillements des procédés de brassage sur la structure de laits fermentés STELA Colloquium, Quebec City, Canada June 1–2
- Lussier, N., D. St-Gelais, S. Villeneuve, S.L. Turgeon 2015 Rôle de la teneur en matières grasses et du type de brassage sur l'évolution des propriétés rhéologiques du yogourt. STELA Colloquium, Quebec City, Canada, June 1–2

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



Improving fodder grass in the context of climate change

Duration : 2014 –2017

Highlights

Context:

- 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.

Anticipated results:

- The best grass in association with alfalfa in Quebec's growing conditions;
- The consumption of tall fescue by animals and animal performance when the fescue is preserved in the form of wilted or semi-dry silage in comparison with Timothy grass;
- The best grass-alfalfa binary association from a technical-economic and environmental point of view when the dairy farm is considered as the only decision-making unit;
- The development of Timothy grass germoplasms with the best regrowth rate.

Potential benefits:

- Increased knowledge about fodder species that will best allow dairy producers in Quebec to make more informed choices.
- The results of this optimization study, based on a global farm model, will help to confirm the most profitable strategy for dairy producers and to quantify the environmental impact of the choices made.
- The identification of Timothy grass populations with the best regrowth rates allow for the development of new plant varieties.
- Ultimately, this project will improve adaptation to 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 results anticipated at the end of the project are: 1) the grass with the best association with alfalfa in Quebec's growing conditions; 2) animal consumption of tall fescue and animal performance when the fescue is preserved in the form of wilted or semi-dry silage in comparison with Timothy grass; 3) the best grass-alfalfa binary association from a technical-economic and environmental point of view when the dairy farm is considered as the only decision-making unit; 4) the development of Timothy grass germoplasms with the fastest regrowth rates. Increased knowledge about fodder species that will best allow dairy producers in Quebec to make more informed choices. It will

be possible to develop recommendations for choosing plant species as well as how they should be managed. The results of this optimization study, based on a global farm model, will help to confirm the most profitable strategy for dairy producers and to quantify the environmental impact of the choices made. The genetic selection of Timothy grass will allow for the development of a population with a faster regrowth rate, which will also make it possible to develop new plant varieties. Ultimately, this project will improve adaptation to climate change. Most of the results will be able to be used directly on farms since fodder crop management is essential to a dairy farm's success.

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Robert Berthiaume
Valacta

Professionals trained

Three students will be trained at the masters and doctoral levels within the framework of this research project:

Florence Pomerleau-Lacasse is currently an undergraduate student at McGill University. She has participated in plant experimentation monitoring in the summer of 2015 and will again participate in summer 2016. She will be enrolling as a master's student in the Department of Plant Science in 2016. She will then be responsible for the fodder grass plot testing component of the project.

Anne-Marie Richard began her master's in animal science at Université Laval in winter 2016. She is responsible for the animal testing component of the project and is 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 at Université Laval. She is responsible for the modelling component of the project. These results will determine the economic and environmental impacts on the farm as a whole, as well as the practices that will be proposed as part of this 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

Novalait
Research catalyst

Catalyseur de recherche

Impact of production processes on the microbiologic quality of Greek yogurt and the valorization of bi-products

Duration : 2014 – 2017

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 by-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 environmentally friendly design of the new process will provide a simple and cost-effective biological recycling option to produce high value-added polysaccharides.

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 by-products in the production of HPC yogurt.
- Hypothesis 1: the protein content enrichment method may modify the conditions that are conducive to the stability, survival and activity of the microbial community during the production and storage of high protein content (HPC) "Greek-type" yogurt.
- Hypothesis 2: the by-products of production may be valorized by obtaining ingredients containing prebiotics.

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.
 - Better understanding of the growth and stability of probiotics in HPC yogurts.
 - Better understanding of the process on contaminant survival.
- Development of high value-added ingredients using by-products.
 - Better control of the co-culture of lactic acid bacteria and yeast in the whey permeate.
 - Better understanding of gene expression in microorganisms during fermentation.

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

Potential Benefits

- Economic:
 - 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)
- Environmental:
 - Decreased energy use and reduced loss of co-products.
- Social:
 - Improved functionality of dairy products for consumer health.

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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 (i) food processing through the use of microorganisms such as lactic acid bacteria and yeasts (ex: fermentation); (ii) the development of foods that are beneficial to health, specifically through the integration of probiotics; (iii) combatting pathogens and microbial contaminants; (iv) improving food safety and preservation; and (v) the valorization of by-products resulting from food processing (ex: lactic acid bacteria).

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-qPCR, and the valorization of food industry by-products such as whey ultrafiltrate. 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.

For further information

Results will be shared through written communication (theses and articles), as well as through oral presentations and posters.

The events planned for 2016 include:

- Novalait Forum (June 2, 2016, Drummondville): poster will be presented by Andréanne Moineau-Jean.

- IUFoST 18th World Congress of Food Science and Technology (August 22–25, 2016, Dublin, Ireland): poster will be presented by Andréanne Moineau-Jean.

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

Novalait
Research catalyst

Systems biology applied to cheddar production

Duration : 2014 –2017

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.
- This project is divided into five objectives.

Objectives

- Obj. #1. Determine the microbiome and virome of milk and cheddar.
- Obj. #2. Determine the microbial and viral transcriptome of milk and cheddar.
- Obj. #3. Determine the microbial and viral proteome of milk and cheddar.
- Obj. #4. Determine the metabolome of milk and cheddar.
- Obj. #5. Determine the biology of the cheddar system. This inclusive objective will allow us to pinpoint 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

Results and potential benefits

- OBJECTIVE #1: Currently, eighteen batches of cheddar are being monitored (after 4 days of production, after 3 months, 6 months and 18 months). To date, the milk and cheese samples that were sampled at 4 days and 3 months have been received, and biochemical and organoleptic analyses have been completed. A protocol has been adapted to separate bacteria and bacteriophages from the dairy matrices. The starter cultures used have been obtained and their genomes are in the process of being sequenced. We have also developed a database containing the DNA sequences of the microbial species recognized as being part of the microbiome of various cheeses.
- OBJECTIVE #2: We have not yet begun work on this objective.
- OBJECTIVE #3: Currently, our extraction and detection methods by LC/MS-MS detect 80% of viral proteins and 57% of bacterial proteins.
- OBJECTIVE #4: We have developed a protocol for the extraction and analysis through LC/MS-MS of the metabolites contained in cheese using an ionic mobility cell. Our preliminary tests showed a high level of metabolic diversity in the samples analyzed. For example, we have detected 8,000 ions as potential metabolites contained in cheddar.
- OBJECTIVE #5: We have not yet begun work on this overarching objective. 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.

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Novalait
Research catalyst

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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 three ways:

- The knowledge acquired, as well as a user-friendly bioinformatic tool, will be made directly available to dairy producers through meetings with members of the Novalait steering committee and during the Novalait Technological Forum.
- We also plan to publish our results in respected international journals with the review committee (for example, Appl. Environ. Microbiol.).
- The students will also have the opportunity to share their results at international conferences such as the annual General Meeting of the American Society for Microbiology and the triennial Symposium on Lactic Acid Bacteria which will take place in 2017 in the Netherlands.

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

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

Duration : 2014 – 2017

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 allows 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

Preliminary observations:

- 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.
- However, the energy required for the heat treatment of UF-YOG is greater than for UF-MILK yogurt.
- A comparative Life Cycle Assessment (LCA) of the two processes concluded that the environmental balance for the entire life cycle presents disadvantages for the UF-MILK process in comparison to the traditional ultrafiltration (YOG) process due to the larger initial quantity of milk required. Milk production is responsible for approximately 80% of the impacts on the life cycle of Greek yogurt.

- The volumic concentration used to concentrate the milk has a direct effect on the environmental impact of Greek yogurt.
- These conclusions must nevertheless be expanded upon in regards to the valorization potential for sweet whey permeate resulting from the UF-MILK process in comparison to acidic permeate. (The impacts of the process may in part be allocated to the co-products of the UF permeate, reducing those of yogurt.)

Perspectives:

- The classical analysis of mass balance and environmental impacts (LCA) will allow for the development of an analysis chart that industrial dairy producers will be able to use in order to optimize their processes while improving their energy efficiency.

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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.

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Novalait

Poster Session Forum Techno 2016

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* CDC Scholarship Program



Milk enriched with omega-3 fatty acids: The challenges of conservation

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Summary

Context.

Increasing the content of ω -3 fatty acids (FA) in milk is accompanied by a concomitant increase in sensitivity to fat oxidation. However, potentially antioxidant compounds occur naturally in some foods used in the dairy cow diet.

Approach.

-To evaluate the evolution of oxidative sensitivity of milk via the administration of increasing doses of flaxseed oil infusion in the abomasum.

-To determine the transfer of the antioxidant power of a concentrated extract of alfalfa as a source of carotenoids, and of flaxseed meal as a source of lignans, in the milk of cow having flaxseed oil infusion in the abomasum.

Results and applications.

Our studies have first verified that the susceptibility to oxidation of the milk (peroxidation index and concentration of several oxidation products) increased linearly as a function of its content in unsaturated FA. The evaluation of the oxidative stability of the milk indicated that the addition of alfalfa concentrated extract in the

ration reduces the redox potential of the milk, as well as its content in FA oxidation products compared with the negative control without antioxidant. However, the oxidative stability of milk has not been improved by the addition of flaxseed meal as a source of lignans in the ration. Our work has shown that the milk enriched with ω -3 AG to the levels required for nutrition labeling according to Canadian standards was a product very vulnerable to oxidative damage. Although the extract of alfalfa has shown some efficiency in preventing this undesirable process, further research is needed to identify foods with higher antioxidant potential.

Potential benefits.

Ultimately, the results of this research will enable losses to be reduced by decreasing decommissioning of milk due to its spontaneous oxidation. This work could contribute to improve the quality of dairy products by modulating the FA profile of milk fat while maintaining its integrity, but also by increasing its content of natural antioxidants.



A new ingredient to maximise omega-3 content

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Summary

Context.

In Canada, the market for ω -3 fatty acids is growing. However, it is difficult for dairy farmers to get into this niche market. Indeed, whole milk normally contains less than 60 mg of ω -3 fatty acids per 250 ml, while the Canadian standards require an intake of 300 mg per reference quantity for identifying a product as a "source of ω -3 fatty acids". The difference between the milk produced in the current conditions of farming and Canadian standards is substantial. The means available to producers to increase the ω -3 content in milk are varied, but do little to achieve the labeling standards. In this regard, the saponification of fats, causing calcium salt formation, has been proposed as a mean to protect the unsaturated fatty acids during the digestive process in the cow. Calcium salts are already commercially available and their industrial production leads to a mixture of particles whose particle size is variable.

Approach.

Calcium salts of fatty acids were screened to separate the fine and

coarse particles. These two fractions were administered to cows and compared with unsaponified lipids to measure the effects on production capacity and efficiency of ω -3 fatty acid transfer in milk fat.

Results and applications.

The fat production and the fat content were higher for cows fed on coarse calcium salts compared to the same amount of unsaponified lipids. The content of ω -3 fatty acids was three times higher in milk fat of cows eating coarse calcium salts. This enabled the production of a whole milk which contained 252 mg of ω -3 fatty acids per serving which is close to the Canadian standards for nutrition labeling.

Potential benefits.

Further work is needed to optimize this technology. However, the results allowed us to consider the marketing of milk enriched with ω -3 fatty acids, produced on the farm, and thus to develop a new range of niche products for the dairy sector.



Potential use of odd and branched chain fatty acids as a diagnostic tool

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Summary

Context.

Odd and branched chain fatty acids (OBCFA) are found in milk and are derivatives of lipid synthesis by ruminal microorganisms. Some studies have shown that the OBCFA profile of the milk could be used as a tool to estimate the efficiency of rumen fermentation. Furthermore, the FA profile of milk varies depending on the stage of lactation. This profile is also influenced in various ways by the addition of lipid supplements to the ration. However the impact of these factors on the relationship between the OBCFA profile of the milk and the environmental conditions of the rumen is unclear.

Approach.

- To evaluate the effects of lactation stage on the secretion of OBCFA in milk related to the mobilization and the restoration of body fat reserves.
- To determine the effects of different lipid supplements on OBCFA synthesis by rumen microbes and the secretion of these fatty acids in milk.
- To check the changes in OBCFA profile in milk following the administration of an acid-producing ration.

Results and applications.

Some OBCFA are found in the adipose tissue of cows. The mobilization of body reserves in early lactation leads to the release of these OBCFA from the adipose tissue, thus explaining their increase in milk fat. Similarly, lipid supplements influence the secretion of milk OBCFA. These phenomena must be considered in researches made to establish relationships between milk OBCFA content and rumen function. Following the administration of an acid-producing ration, some OBCFA evolve similarly, suggesting a common microbial origin. It is therefore appropriate to group some OBCFA, simplifying analysis and improving the robustness of established relationships. The results have made possible the development of equations to evaluate the balance in the rumen from milk FA profile, regardless of lipid supplementation.

Potential benefits.

This research will affect dairy herd feeding management by offering additional tools to better predict the nutritional value of rations. These results will also allow the rapid diagnosis of metabolic disorders such as subclinical ruminal acidosis.

Metagenomic analysis of industrial membranes biofilms

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Summary

Pressure-driven membrane processes have revolutionized the dairy industry by facilitating the valorization of its by-products. However, their performances are still affected by long-term fouling phenomena. It is hypothesized that the formation of microbial biofilms surviving to daily cleaning and sanitizing cycles lead to long-term flux decline due to the production of extracellular polymeric substances. Moreover, these bacterial communities may represent a serious hygiene concern in filtration systems. There are currently no analytical method enabling the identification of bacteria on membrane surfaces used in the dairy industry. Our study was conducted to design a targeted metagenomic tool in order to establish a global portrait of bacterial communities on membranes. A total of seven (7) industrial spiral-wound elements from four (4) dairy processing plants of Quebec were sampled at the end of their useful lifetime. Targeted analysis of the genomic DNA (16S rRNA gene) extracted on membranes unraveled the bacterial diversity found on them via high-throughput sequencing technology (Illumina MiSeq). It was found that

the nature of the fluid filtered (milk, whey, water) explains 58.6% of the variance observed ($p < 0.001$) between communities found on membranes. It appeared that some treatments applied on dairy fluids (milk pasteurization, whey bleaching or whey ultrafiltration) impose a selective pressure on bacteria in the filtered fluids. It affects the diversity of bacterial communities found on membranes and the proportions of spore-former bacteria (known to be more resistant) that increase when membranes are used to filter fluids that undergo a higher number of treatments. This work provides first experimental data that will help in designing preventive measures and cleaning strategies specifically designed to prevent biofouling in dairy processing plants. The analysis tool developed for this study will permit, in a medium-term objective, to define optimal operating parameters, including cleaning and sanitizing, that promote the growth of desirable bacteria over unwanted ones. It will result more efficient systems producing more valuable dairy products.

Milk microfiltration on spiral-wound polymeric membrane : impact of pore diameter

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Summary

Microfiltration (MF) using 0.1 and 0.2 μm pore diameter membranes, allows the separation of the soluble and colloidal phases of milk generating a retentate enriched in casein (CN) for use in cheese processing and a permeate rich in serum proteins (PS) with high added value. MF of milk is generally performed using ceramic membranes, thus a few studies have investigated the use polymeric membranes to date. However, in the context of eco-efficiency, MF using spiral-wound polymeric membranes proves to be beneficial in terms of investment costs and better performance. The main objective was to assess the impact of transmembrane pressure (TMP) change on the rejection of CN and transmission of PS by MF of pasteurized skim milk using spiral-wound polymeric PVDF membranes of 0.1 and 0.2 μm pore diameter to identify the optimal separation conditions. The experiments were repeated 3 times each on a full-recycle mode at 50°C with TMP of 89.6, 106.9 and 124.1 kPa. Protein content was measured using total nitrogen, non-casein, and non-

protein by Kjeldahl method and protein characterization (SDS-PAGE) was carried out to quantify the separative performance. Results showed that the membrane's pore diameter had no significant impact ($P>0.05$) on the permeate flux, but that the TMP increase generated a steady increase of permeate flux for the two membranes. Regarding protein separation performance, the 0.1 μm membrane retains significantly ($P<0.05$) more CN and PS compared to the 0.2 μm membrane. Furthermore, for the two membranes, the TMP did not have any significant impact ($P>0.05$) on the rejection of CN and PS. Preliminary results show that it is possible to separate CN from PS with spiral-wound polymeric membranes. In addition, better separative performance was observed for the 0.1 μm membrane. The use of this type of membrane, advantageous economically, would be an interesting choice for the dairy industry milk to concentrate CN in milk. However, it will be necessary to study their performance during milk concentration experiments.



Views of dairy producers, veterinarians, and farm advisors on culling rate

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Summary

The culling decision is part of the dairy producer strategy to minimize losses. But when it's time to decide an animal have to leave the herd, the producer takes into account several parameters like, for example, constraints about milk quality, production quota, available spaces in the barn etc. It was already demonstrated that dairy producers objectives, motivations and performances are more influenced by sociological, cultural, and psychological factors than by animal or economic sciences. Researches on motivational and behavioural aspects of farmers' decision utility are sparse, and nonexistent regarding culling expectations and the associated decision process. Culling decision criteria are usually postulated from the researcher point-of-view but were never described from the decision-maker point-of-view. Our goal was to identify shared criteria on culling decision held by dairy producers under preventive medicine program, veterinarians, and farm advisors.

A common profile was identified for producers, veterinarians, and advisors. This profile shows the importance given by dairy producers to udder health and to produce a healthy, secure milk. Milk production level is also a strong element taken into account. General factors related to farm management like debt level, workforce, material or building amortization, or milking parlour capacity are not considered. To this general common shared profile, two subgroups of advisors showed a particular profile. One includes as prime decision determinants cow's reproductive status and management of animal flow (in: heifers vs. out: culled cows), while the other is concerned about animal welfare. The information gathered during this study allow to better understand why and how the various actors are taking the culling decision, and to evaluate potential obstacles to a better collaboration between some actors and dairy producers.



Mineral Nutrition: New perspective for optimal milk production

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Summary

Context.

The high proportions of concentrates incorporated into rations to support the needs of high-yielding cows often induce ruminal disturbances. Subclinical ruminal acidosis is also identified as a risk factor associated with the milk fat drop syndrome. Studies have shown the possibility that an increase in food cation-anion difference (DCAD) and/or adding a K_2CO_3 supplement to the ration can prevent changes in ruminal biohydrogenation pathways associated with diets high in concentrate, which have inhibitory effects on the synthesis of milk fat.

Approach.

- to distinguish the effects of DCAD, of the K content and of the buffering capacity of the ration on the biohydrogenation in the rumen and the milk fat content.
- to check the interaction between the intakes of K_2CO_3 and soybean oil on:
 - a) the rumen microbial populations;
 - b) the biohydrogenation of fatty acids;
 - c) the performances of the animals.

Results and applications.

There is great variability among cows in the ability of their rumen

to resist to rations rich in concentrates. Furthermore, the addition of K_2CO_3 in the ration leads to an increase of bacteria known for their role in the biohydrogenation of lipids. However these variations have no impact on the synthesis of milk fat. By comparing the effects of different sources of K in the ration on the mineral profile of milk, we found that increasing K contents of the ration via the addition of K_2CO_3 causes ion imbalance in the mammary epithelial cell which results in a decline in milk production. Finally, there is a significant correlation between the Cl concentration of milk and the milk production, regardless of ration treatments.

Potential benefits.

These innovative results lead us to investigate the impact of mineral food on the functioning of mammary tissue. The next steps will enable us to identify an ideal mineral profile that first will contribute to maintaining the balance of the rumen, and second to promote the ability of the mammary cell to capture the necessary nutrients to milk synthesis.



Alternatives grasses to timothy in association with alfalfa

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Summary

In Quebec, the main grass species cultivated with alfalfa is timothy, which has a limited regrowth potential under dry and warm summer conditions. This project aims at finding alternative to timothy in the context of climate change. The objective is to compare six grass species in binary association with alfalfa at three contrasting sites in Quebec (Ste-Anne-de-Bellevue, St-Augustin-de-Desmaures, Normandin). Six binary associations of alfalfa with timothy, tall fescue, meadow fescue, festulolium, perennial ryegrass and meadow brome were seeded in 2014 and harvested in 2015, either at early bud or early flower stages of alfalfa. The total yield and yield contribution of the different botanical components were measured at each cut. An additional harvest year will be performed in 2016, and the nutritive value of forages harvested in 2015 and 2016 will be measured. Festulolium and perennial ryegrass with alfalfa

had a lower seasonal yield than other associations. Alfalfa, grass and weed yield contributions were highly variable for the six mixtures. In addition, the alfalfa developmental stage did not significantly affect the grass yield contribution or the seasonal yield, even though there was an additional cut performed at the early bud stage. These preliminary results suggest that tall fescue, meadow fescue and meadow brome have a similar or superior productivity than timothy, and they confirm winter survival issues of festulolium and perennial ryegrass at the northernmost site. However, results from a second harvest year will be necessary to conclude. This project will allow the identification of alternatives to timothy cultivated with alfalfa in order to maximize the productivity, nutritive value and persistence of this type of forage mixture in the context of climate change.

A real-time PCR (qPCR) method for the quantification of indigenous yeast in the cheese ecosystem

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Summary

The complex fungal ecosystems of specialty cheeses are increasingly studied because of the potential contribution of indigenous yeasts to the development of the cheese's sensory properties. Previous studies detected *Cyberlindnera jadinii*, *Kazachstania servazzii* and *Pichia kudriavzevii* in both raw milk and/or artisanal specialty cheeses from the province of Quebec. Based on these results, we developed a highly specific and sensitive real-time quantitative PCR (qPCR) assay to quantitate these yeast species. For each species, primers and TaqMan probes were designed based on the DNA sequence of a specific target gene. Genes encoding the malic enzyme, centromere H3 protein and glutathione S-transferase were selected for *C. jadinii*, *K. servazzii* and *P. kudriavzevii*, respectively. The specificity of the qPCR assays was validated and showed no cross-amplification using 11 other fungal

microorganisms usually found in bloomy-rind and smear-ripened cheeses, such as *Geotrichum candidum* and *Debaryomyces hansenii*. Our results showed a high PCR efficiency, ranging from 90% to 105%, with r^2 -values of > 0.99 . These qPCR assays are efficient for quantifying the incidence of *C. jadinii*, *K. servazzii* and *P. kudriavzevii* in various specialty cheeses (bloomy- and smear-cheeses from raw, thermized and pasteurized milks). Tracking indigenous yeasts allows a better understanding of yeast diversity in cheese according to milk treatment, type of rind and region of production. This study is the first step in better understanding the possible participation of these indigenous yeasts in the development of cheese flavors, and their role in the fungal ecosystem.

Impact of buttermilk constituents on the on the rennet coagulation of milk

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Summary

Buttermilk is the by-product obtained from the churning of cream into butter. Its composition is similar to that of skim milk, but its rennet coagulation aptitude is lower. This defect has usually been attributed to the presence of milk fat globule membrane (MFGM) fragments in buttermilk. In addition, pasteurization of cream modifies these fragments that become more likely to affect the coagulation of milk. The objective of the present work was therefore to isolate MFGM fragments from raw (RCB) and pasteurized (PCB) creams buttermilks (BCC) and to determine their impact on rennet gel formation and properties. The isolates containing MFGM fragments were obtained by ultracentrifugation (100,000xg, 1 hour, 34 ° C) of BCC, BCP and raw skim milk (LEC) (control) after casein micelles dissociation with sodium citrate (2% w /v). The pellets were then washed with milk ultrafiltration permeate to remove sodium citrate. The protein composition of the substrates and the isolates was determined by Kjeldahl and gel electrophoresis (SDS-PAGE). The isolates were re-dispersed in reconstituted skim milk

enriched to 3.5% protein to determine their impact on milk coagulation kinetics and gel syneresis at cooking. In all isolates, a little more than 50% of the proteins were > 35 kDa consisting mainly of membrane proteins, but there were 14 to 16 times more protein isolated from buttermilks than from skim milk. The significant presence of serum proteins and casein in isolates of both types of buttermilk suggests that churning results in their association with membrane fragments or induces changes promoting their co-sedimentation. Buttermilk isolates interfere with the formation of rennet gel and reduce its ability to syneresis, but with no impact of cream pasteurization. These new data contribute to knowledge acquisition for a better understanding of the functionality of buttermilk in cheesemaking.

Impact of high pressure of milk homogenization on its facility cheesemaking properties

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Summary

There is a growing interest in the application of high pressure homogenization (HPH) for the treatment of milk and dairy product development. However, the impact of the HPH on cheesemaking properties of milk has not yet been studied. The HPH modifies the structural properties of casein micelles, which may thus influence milk coagulation. The aim of this study was to determine the effects of skim milk HPH on its cheesemaking ability. Raw skim milk was homogenized at four different pressures (0, 50, 100, 150 MPa) with one or three passes through the homogenizing valve. The size of the casein micelles and their zeta potential were determined by dynamic light scattering. The profile of casein and calcium content in milk soluble phase were determined by reverse phase HPLC and ICP-OES respectively. The coagulation properties of homogenized milk after renneting were measured by determining the

kinetics of caseinomacropeptide (CMP) release, the change in optical density and the contraction of gels during cooking. Model cheeses were also made to assess protein, fat retention and cheese yield. The results showed a significant decrease ($p < 0.05$) in the size of casein micelles and their net negative charge according to the homogenizing pressure. The proportion of κ -casein and the calcium concentration in the soluble fraction significantly increased ($p < 0.05$) with increasing the homogenization pressure. These transfers from the colloidal to the soluble phase of milk suggest partial disintegration of casein micelles. However, these structural changes of casein micelles had no impact on the cheesemaking properties of milk. Therefore, skim milk homogenization up to a pressure of 150 MPa cannot be used to improve the cheesemaking properties of skim milk or cheese mass balance.

Impact of stirring, smoothing and cooling on the rheological properties of stirred yogurt in industrial simulation

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Summary

A limited number of studies have considered the quality of stirred yogurt, but at the laboratory scale only. The goal of this project was to evaluate the effect of processing parameters such as stirring, smoothing and the cooling system on the rheological properties of yogurt using a pilot-scale unit simulating the industrial process. Indeed, after the fermentation process, these operations disrupt the protein matrix and affect the quality of yogurt. A dairy mix was standardized to 14% total solids, 0% fat and 4% protein, homogenized, heated (94.5°C, 5 min) and inoculated at 41°C using lactic acid bacteria. Two stirring times (5 or 10 min), two cooling systems (plate or tubular heat exchanger) and two smoothing temperatures (35°C or 20°C) were tested. Samples were collected at different positions to determine the individual impact of the operations. Stirred yogurts were analyzed after 1, 3, 13 and 22 days of storage at 4°C. Syneresis (centrifuge), apparent viscosity (rheometer), firmness (texturometer), consistency (Bostwick consistometer) and flow behaviour (Posthumus funnel) were analyzed. During processing, the effects of heat exchangers type and smoothing temperature were more

marked than the effect of stirring on the augmentation of viscosity and the reduction of syneresis, firmness and consistency of the stirred yogurt. During storage, however, heat exchangers had a major impact on the product's quality parameters. Using a plate heat exchanger resulted in reduced syneresis, firmness, viscosity and consistency in comparison with the use of a tubular heat exchanger. A smoothing temperature of 35°C increased the flow time whereas stirring had little effect. After 22 days of storage, syneresis decreased but viscosity, firmness, consistency and flow time increased. The pilot-scale unit allowed the simulation of industrial process and helped provide better knowledge of the individual and cumulative effects of unit operations on the quality parameters of yogurt. In the long term, the results of this study will be useful for reducing low-quality products and economic losses. In the short term, the Bostwick consistometer and the Posthumus funnel methods can be recommended to the yogurt industry as quick and easy means of determining the firmness and viscosity of stirred yogurt.

Effect of ingredients and the shearing temperature on the structure and properties of stirred yogurt

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Summary

The production of stirred yogurt in Canada is continuously increasing. However, if the interactions between formulation/processes for firm yogurt are well documented in the literature, this is not the case for stirred yogurt because it is difficult to reproduce in the laboratory, the industrial mixing steps. During the fermentation step, the gel is forming. Denatured whey proteins increase resistance to syneresis and the textural attributes of the dairy gel. The conditioning steps involved in the production of stirred yogurt, shear and break the gel, alter the microstructure and the rheological properties of the yogurt. In this work, a rheometer simulated the shearing step of yoghurt by controlling shear rate and temperature. Identical formulations (total solids, protein content and ratio of casein/whey protein) containing whey protein isolate (WPI) or whey protein concentrate (WPC) were compared. Yogurt has been smoothed according to one of the following temperature profiles: 42 °C (Y42), 20 °C (Y20) or a ramp from 42 to 20 °C (YR). Forced syneresis was

measured three days later. The particle size was measured on days 0, 4 and 7 by laser diffraction and optical microscopy followed by image analysis. For Y42, syneresis was high regardless of the protein ingredient. The images showed an heterogeneous network (serum separated from the gel) for Y42 while for Y20 and YR it was homogeneous. Either by image analysis or laser diffraction, the shearing temperature modulates the particle size; ranging in ascending order for Y20, YR, Y42. Only the image analysis revealed an increase in the particle size between day 0 and day 4 after production; indicating a reorganization of the particles in the first days of storage. The ingredient used had little influence on the properties studied. To optimize the cost and the quality of yogurts, cooling before smoothing is imperative to minimize graininess and syneresis. However, there would be no harm in using a less pure source of whey proteins to adjust the casein/whey protein ratio (eg WPC 35%) to reduce production costs.

Effect of fat content and industrial shearing on the rheological properties of stirred yogurts

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Summary

Most of the yogurt produced in Canada is stirred yogurt. After being fermented in tanks, the yogurt is stirred, causing the structure breakdown of the protein matrix. However, few studies have demonstrated the effect of industrial stirring, since it is difficult to simulate industrial conditions in a laboratory setting. The purpose of this project was to determine the impact of fermentation time and two industrial cooling systems on the rheological properties of stirred yogurt with different total solids and fat content. The yogurts were standardized at 16.5% TS, 4% protein, and fat content ranging from 0.0% to 3.9%. The fermentation times were 3 h, 4 h and 5 h. A fat-free control was produced at 14% total solids. All of the yogurts were stirred using a pilot-scale unit and cooled using a tubular heat exchanger (low-shear) or a plate heat exchanger (high-shear). The physicochemical (syneresis index), textural (firmness) and rheological (apparent viscosity) properties were analyzed after 1 d and 34 d stored at 4 °C. Increasing total solids reduced syneresis but

had no impact on firmness or viscosity. Increasing fat content reduced syneresis and increased firmness and viscosity. A fermentation time of 3 h seemed to produce yogurts with lower firmness and viscosity and higher syneresis than a fermentation time of 4 h or 5 h. The use of tubular heat exchanger cooling produced firmer yogurts with higher syneresis than plate heat exchanger cooling. However, the type of cooling had no impact on viscosity. During storage, firmness and viscosity increased, while syneresis remained stable. The use of the pilot-scale unit allowed determining the impact of the two types of industrial cooling systems on changes in the rheological properties of stirred yogurts with different total solids and fat contents. This new knowledge should enable manufacturers to better identify the factor that has the greatest impact during the production of stirred yogurts and thus to reduce product downgrading in the long term.



Clinical trial of a milking protocol in early lactation: effect on ketone bodies in the blood

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Summary

The hyperketonemia, with or without clinical signs, is a metabolic disease which limits milk production, reproductive performance and increases the risk of diseases and culling. The temporary reduction of the energy demand to limit the fat mobilization in early lactation will reduce its incidence. This study evaluates the impact of a protocol that involves incomplete milking (IMP) in early lactation on blood ketonemia and on hyperketonemia of multiparous cows in commercial context. From December 2013 to March 2015, 846 Holstein cows from thirteen herds were randomly assigned, before calving, to one of two treatments: 1- complete milking procedure (CMP) or 2- IMP (milking limited to 10-14 L of milk per day for the first five days in milk; DIM). Once a week between 1 to 26 DIM, some blood was taken to assess serum β -hydroxybutyrate (BHBA), a ketone body marker of negative energy balance. For the period

between 4 to 7 DIM, the serum concentration of BHBA of cows exposed to the IMP was 17% lower than that of the cows on the CMP ($P < 0.01$). For the period from 8 to 17 DIM, the serum concentration of BHBA of cows exposed to the IMP tended to be 6% lower than that of the cows on the CMP ($P = 0.09$). The risk of hyperketonemia (serum BHBA ≥ 1.4 mmol/L) was reduced for cows on the IMP compared to cows on the CMP. Specifically, the percentage of cows with hyperketonemia between 4 and 7 DIM was 5% for IMP and 11% for CMP ($P = 0.03$). Between 8 and 17 DIM, these percentages were 18% for IMP and 23% for CMP ($P = 0.05$). IMP helps reduce ketonemia and hyperketonemia in early lactation compared to CMP. Easily applicable on the farm, the IMP does not require any medication and some extra equipment. The study will continue to quantify the impact of IMP on welfare, production, reproduction, health and culling.



The impact of incomplete milking in early lactation on cow discomfort

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Summary

Context One potential approach to controlling the negative energy balance of cows in transition is to restrict milk production by making an incomplete milking in early lactation. The objective of this project was to evaluate the effect of incomplete milking on cow comfort and milk losses immediately after milking.

Approach Multiparous cows (n=77) of three commercial herds in Quebec were randomly selected to be either incompletely milked (10-14 L/day) until d 5 after calving (incomplete) or completely milked (control). To evaluate cow comfort and milk flow, three measurements were made: (1) Total number of hours per day lying down during the first two weeks of lactation, (2) Maximum pressure tolerated by cow on the udder before milking during the first week of lactation (algometer applied until the animal shows an avoidance reaction or up to 8 kg force), (3) the presence of milk leaked just after milking during the first week of lactation.

Results The relationship between the number of hours lying and the treatment group varied according to days in milk. During the treatment period (d1-d5), cows incompletely milked were longer lying (10.9 hours/day; n=18 cows) than cows in the control group (9.8 hours/day; n=14 cows). This difference ($p = 0.01$) tend to indicate a greater comfort in the incompletely milked group. The average pressure tolerated by cows on the udder before milking was 6.4 kgf for cows of the incompletely milked group (n=22) and 5.3 kgf in the control group (n=20). This difference was not statistically significant ($p=0.23$). Milk leaked after milking was observed on only one cow incompletely milked (1/23) and two cows in the control group (2/23); this difference was not significant ($p=0.56$).

Benefits As a conclusion, incomplete milking in early lactation does not seem to cause discomfort for dairy cows or milk leaked after milking.

Metabolic engineering for acetoin and butanediol bioproduction from cheese processing byproduct

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Summary

Many researches are conducted around the world to enhance the residual biomass into alternative sources of energy or chemicals. In Quebec, among the industries producing potentially recoverable residues, industrial milk processing generates significant amounts of whey and permeate. Our project aims to assess the possibility of creating, by metabolic engineering, microorganisms capable of producing molecules of commercial interest from lactose content in these substances. Our proof of concept aims at obtaining 2,3-butanediol (BD) by means of a fermentation process which implements an *Escherichia coli* genetically modified. BD is a substance with many industrial applications, including the manufacture of polymers, explosives, pharmaceuticals. Since enzymes necessary for the production of BD are not found in *E. coli*, we first identified three gene products successively to transform

pyruvate from glycolysis in 1) acetolactate then 2) acetoin and finally 3) BD. These genes were synthesized and then incorporated into the chromosome of *E. coli*. We then confirmed that the strain obtained allowed the production of molecules expected before creating a series of deletions inactivating five fermentation pathways that could potentially compete production of BD. Our results show that the addition of the metabolic pathway coupled to the deletion of some endogenous fermentation pathways of *E. coli* brings better yields. Optimization of fermentation conditions could then maximize production, and potentially offer a process economically viable. Finally, the methods developed in this project could also be applied to the production of a wide variety of other molecules to transform production residues into molecules of interest.

Production of acetoin and 2-3 butanediol by whey fermentation

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Summary

Dairy industry is one of the most important inside the agrifood sector around the world. Whey is a liquid waste issued from the transformation of milk into cheese. Whey presents a high organic load due to its high content of lactose (around 50 g/L whey). For this reason, whey has to be treated before being released in the environment. Another wastewater is the whey permeate, obtained after separating the proteins from the whey. Whey permeate has a lactose concentration higher than 80 g/L. The aim of the study is to valorize whey and whey permeate into useful bioproducts and, to reduce their environmental impacts. According to the 2015 Paris Climate Conference (COP 21), 195 governments committed to reduce greenhouse gas (GHG) emissions. Our project could participate in this effort because biofuels or bioproducts could be derived from the whey or whey permeate and replace fossil fuels. For this purpose, whey and whey permeate were fermented in the presence of a new genetically modified strain of *Escherichia coli* MG1655 able to break directly the lactose and convert it into diols (acetoin and 2,3-butanediol). A series of experiments tested the effect of different concentrations of glucose and

lactose, the pH, the anaerobic condition, the nitrogen sources and concentrations and the metal ions on the diols yield in a defined culture medium (M9). These results of these experiments were used to establish the best operating conditions to ferment whey and whey permeate. In this way, the fermentation was performed at a pH 7.4, a temperature of 37°C and an agitation of 100 rpm during 96 hours at laboratory scale using flasks of 500 mL containing 200 mL of working volume (10% (v/v) of inoculum) using urea and metal ions. Under these conditions, diol yields of 21% (g diol/g lactose) for both whey and whey permeate was obtained. The optimization of the operating conditions will be carried out in order to increase the diols yields in subsequent tests at laboratory scale using flasks and a bioreactor of 2 liters. The biovalorization of dairy wastewaters into biofuels would permit to the dairy industry to get new incomes and to become greener.

Environmental analysis of cheese processing byproduct

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Summary

Whey is a by-product of cheese production. In Quebec, in 2010, almost two million tons of whey were generated. The whey consists mainly of water (90-95%) and solid. This solid fraction is mainly composed of lactose (70-73% m/m), proteins and minerals. These compounds can be recovered if they are managed properly. Otherwise, ineffective management of the whey can create pollution (BOD₅ 32,000 mg/L) and can be the source of resources' waste. In order to valorise the lactose in the cheese residue, a new bioprocess named BIOBAC is under development. It consists in the production of chemical compounds of interest in particular diol

(butanediol and acetoin) that are currently obtained from fossil fuels. The objective of this study is to verify the feasibility of this new bioprocess in an environmental perspective using a lifecycle analysis (LCA). The LCA approach includes the quantification and comparison of the environmental impact of various whey processing methods. This comparison will be a useful tool to inform the cheese industry on waste recovery methods. Preliminary results have shown that BIOBAC is an interesting approach from an environmental point of view, since it bioprocess will reduce the current society dependence on oil.

CRISPR-Cas9 for the genome editing and the study of virulent genes of phage p2

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Summary

Bacteriophages (or phages) are bacterial viruses found in every ecosystem. In the milk industry, virulent phages can interfere with milk transformation processes since they are natural inhabitant of milk and they survive pasteurization. Different strains of *Lactococcus lactis* are used in mesophilic starter cultures to produce an array of cheeses, and many of those strains are sensitive to phage infection. Phage p2 is a model for the study of lactococcal phages since it belongs to the most predominant group (936) of phage in Quebec and across the globe. In order to control these phages, we have to gain knowledge on their biology and evolution. Despite its importance, almost half of the genes of phage p2, approximately twenty, encode for proteins of unknown functions. One of the ways to study genes/proteins is to modify them and observe the resulting phenotypes. The study of virulent phages poses a significant challenge because the modification of their genome is limited to the short infection cycle within a cellular host. The main objective of this

project was the adaptation of a gene-editing tool based on the CRISPR-Cas9 technology to study the virulent phage p2. This technology requires Cas9, a protein that can be programmed to cleave a precise genomic sequence when paired with a CRISPR region, which serves as a versatile guide RNA. Over the last year, we adapted this technology in *L. lactis* and it is now functional to inactivate, mutate or tag every gene and protein of interest in phage p2. This innovative tool will enable us to study this phage biology to an unprecedented level and is promising for the advancement of knowledge regarding phage-bacteria interactions. For instance, we believe that some phage genes are responsible for the specific infection of some starter cultures. Hence, this gain of knowledge will be of great value to improve phage control, by allowing a better selection of starter cultures. Furthermore, our work will help minimizing the losses associated with off-grade milk products.

Systems biology applied to cheddar

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Summary

The province of Quebec is the largest producer of cheddar cheese in Canada. Despite this expertise, obtaining constant high-quality cheese products still poses a challenge. Variation in the organoleptic properties of the cheese may occur from one batch to another. Many factors are known to influence this variability, including: the chemical and microbial composition of the milk, the added starter cultures and the presence of virulent phages. This Novalait project, which started in September 2014, aims to study those parameters using an integrated systems biology approach to better understand the impact of the microbial network during the manufacture and aging of cheddar cheeses. Milk samples will be collected after heat treatment while cheese samples will be sampled 1 day after production as well as after 3 months, 6 months and 18 months of ripening. Using Illumina sequencing technology, viral and microbial succession will be examined on all milk and cheese samples from several production lots. The genome of the mesophilic starter cultures used to make the cheeses will also be sequenced. These same milk and cheese

samples will also undergo biochemical analyses. In addition, we are also in the process of adapting a protocol to separate the viral fraction from these dairy samples. After sequencing the bacterial and the viral fractions, the expression of selected abundant genes will be quantified by RT-qPCR. Then, the presence of targeted bacterial and phage proteins will be investigated using proteomics. In parallel, the metabolome of the dairy samples will also be analyzed by mass spectrometry. We are currently developing a protocol to extract and then analyse metabolites from milk and cheese sample using LC-MS/MS and an ion mobility cell. The final objective of this project will aim to establish trends during cheese maturation and identify certain markers of quality to model this cheese biological system. Moreover, obtaining an inventory of microorganisms from milk and at various stages of cheddar ripening will provide a competitive edge to Quebec cheesemakers. Finally, the project could lead to cheeses with superior and more consistent organoleptic qualities.



Transcriptomic analysis of bovine embryos obtained from peri-pubertal donors

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Summary

Assisted reproduction technologies (ART), genomics and high selection pressure observed in the dairy industry are leading towards the use of younger females for reproduction purpose, reducing the interval between generations. This situation might have an impact on embryo quality, which can affect the success rate of the procedures and, potentially, the heifer offspring. This study aims to document the effect of donor age on embryo quality based on transcriptomic pattern to develop tools that will minimize the impact of age and to characterize the effect of using young animals for reproduction purpose. 10 young Holstein cows were used 3 times each at different ages for ovarian stimulation protocols and oocyte collections (at 8, 11 and 14 months). These oocytes were then fertilized *in vitro* with adult bull semen, generating 3 lots of embryo per animal. Semen from the same bull was used for all females, at all times. Each animal was used as its own control for age-effect evaluation. EmbryoGENE platform allowed whole genome assessment of gene expression patterns at the

blastocyst stage. Age-related *microarray* contrast analysis (8 vs 14 months and 11 vs 14 months) allowed to identify 242 differentially expressed genes (DEGs) for the first contrast and 296 for the second. The analysis of the functions of the DEGs suggest a metabolic cause to explain the differences that are observed between immature and adult subjects, leading to an higher impact of *in vitro* conditions in the blastocysts from younger heifers. In fact, amongst the gene expression pathways affected by age, bioinformatic has highlighted the importance of the mTOR and PPAR signaling pathways as well as the NRF2-mediated oxidative stress response pathway. This study is the first to demonstrate the effect of donor age on bovine embryo quality by transcriptomic analysis, using peri-pubertal commercial stimulated Holstein subjects. The main conclusion is that the effect of age is marginal and does not impair seriously the embryo quality. The gene pathways identified may suggest modifications to the heifer nutritional program and embryo culture conditions to mitigate the age effect.



Evaluation of immune response to dry treatment without antibiotic based on chitosan hydrogel

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Summary

The transition from lactation to the dry period in dairy cows is a period of high risk for acquiring new intra-mammary infections. This risk is reduced when the involution of the mammary gland is completed. Accordingly, approaches that accelerate the involution process after drying-off could reduce the incidence of mastitis. Our research program aims at developing a biological response modifier that could be injected into cow teats to promote immune cell migration and speed up mammary involution. Chitosan is a natural polysaccharide derived from chitin which is able to trigger host innate immunity. We have developed two formulations, using crab or shrimp chitosan, which are liquid at room temperature but form a hydrogel at the body temperature. Each quarter of 7 Holstein cows in late lactation was randomly assigned at drying-off to an intra-mammary infusion of 2.5 or 5 mL of crab chitosan hydrogel (crab2.5 or crab5), 5 mL of shrimp chitosan (shp5) or 5 mL

of saline (control). Milk (mammary secretion) samples of each quarter were collected on days before and after drying-off to measure different involution markers. The chitosan hydrogel infusions (crab5, crab2.5 and shp5) have hastened the increases in somatic cell count, serum albumin and lactoferrin concentrations, as well as the lactate dehydrogenase activity in mammary secretions. No major difference between sources or volumes of chitosan was noted for the measured parameters. These results suggest that chitosan hydrogel infusion hastened mammary gland involution, which may reduce the risk of acquiring new intra-mammary infection during the dry-off period. When completed, this approach could be used as an alternative to dry cow antibiotic therapy for non-infected cows.

Impact of Greek-style yogurts rich in proteins on the development of probiotics and storage contaminants

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Summary

Greek-style yogurts are very popular among consumers. As for regular yogurts, probiotic bacteria can be added. However, knowledge is lacking on how the production processes of high protein (HP) yogurts impact their survival. The aim of this project was to compare the survival of two commercial probiotic strains and two microbial contaminants in two HP products during storage. Yogurts with 10% protein content were produced either by ultrafiltration of the milk (HP-UF) prior to fermentation or by centrifugation of a regular yogurt (HP-CF). Regular yogurt (4% protein) was used as control (C). The growth and stability of two probiotic bacteria (sensitive: *Bifidobacterium longum* subsp. *longum* R0175 (R0175) and resistant: *Lactobacillus helveticus* R0052 (R0052)) with an inoculation rate of 1.0×10^7 CFU/g, and two microbial contaminants (*Kluyveromyces marxianus* and a non-pathogenic surrogate of *Escherichia coli* O157:H7) with an inoculation rate of 1.0×10^5 CFU/g, were followed in yogurts during storage at 4°C. The two HP yogurts contained 3 to 7 times more of the

resistant strain R0052 than the control (HP-UF = 1.45×10^8 , HP-CF = 8.02×10^7 , C = 2.10×10^7 CFU/g). The stability during storage of R0052 was greater in the HP-CF yogurt, with 15 times more probiotic after 45 days than in the control (HP-CF = 1.87×10^7 , C = 1.27×10^6 CFU/g). However, the survival of the sensitive probiotic strain R0175 was not improved in the two HP yogurts. On the other hand, the HP-UF yogurt allowed a 2 log CFU/g reduction of *E. coli* viable cells in 27 days, compared to 0.8 log CFU/g reduction for the control. However, the yeast *K. marxianus* was able to grow in the three yogurts during storage (+2 log CFU/g in 45 days). Our results show that a new kind of Greek-style yogurts rich in probiotics can be produced by the dairy industry in a short term perspective. This comparative data ease the choice of manufacturing processes and the selection of probiotic strains to produce healthy HP yogurts, and also decrease the cost of production and the contamination risks with pathogenic bacteria.

